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677. THE EFFECT OF VARIATIONS IN NUTRIENT INTAKE UPON THE YIELD AND COMPOSITION OF MILK

I. VARIATIONS IN THE AMOUNT AND TYPE OF CONCENTRATES FED IN WINTER RATIONS

BY A. W. A. BURT

National Institute for Research in Dairying, University of Reading

(With 3 Figures)

In spite of their considerable economic importance, there is relatively little direct experimental evidence dealing with the relationships between level of feeding and milk production. The earlier experiments were reviewed by Yates, Boyd & Pettit (1), who concluded that feeding in excess of the normal British standards (2) was profitable, and that economic efficiency was maximized at an intake of approximately 2·8 lb. production starch equivalent/10 lb. milk at prices ruling in 1941–42. Since then, the results of large-scale American input-output experiments have been published (3), and reviews of various aspects of the problem have been given by Jawetz (4), Reid (5) and Burt (6); but until recent experiments at the Hannah Dairy Research Institute (7,8), practically no information was available on the effects of variations in level of feeding using rations and systems of management common in Great Britain.

It is generally accepted that the response of milk yield to increasing energy intake, in the presence of adequate supplies of protein and other nutrients, obeys the law of diminishing returns (1,3) and that, in consequence, high levels of feeding will be most profitable in the winter months when the milk : concentrate price ratio is highest. This paper is a report of five experiments which were carried out during the winter feeding periods of 1954–56 to study the response to changes in the concentrate ration of cows receiving typical winter diets of hay and grass silage. A further experiment dealing with individual rates of eating, and factors affecting the response to additional concentrates is described in Part II (9).

EXPERIMENTAL

Material and methods

Eighteen Ayrshire cows were used in each of Exps. 1–3, eighteen Dairy Shorthorns in Exp. 4, and eighteen Friesians in Exp. 5. Three treatments were compared in each experiment. A randomized block design with experimental periods of 4 weeks was used in Exp. 1, and the changeover design of Cochran, Autrey & Cannon (10) with experimental periods of 3 weeks duration in the remaining experiments.

The normal maintenance standards of 6·0 lb. starch equivalent (S.E.) and 0·63 lb. protein equivalent (P.E.)/day for cows of 1000 lb. live weight (2) were used throughout, and the requirements of individual cows calculated by adjusting these quantities in proportion to (liveweight) $^{0.75}$ (11). Production standards of 2·5 lb. S.E. (2) and 0·5 lb. P.E./10 lb.

milk were used. The latter was used in preference to the normally recommended 0·6 lb. P.E./10 lb. milk in view of the well-recognized absence of response to additional amounts of protein (7, 12, 13, 14).

Control periods of 3 weeks were run before each experiment, when rations closely approximating to the normal standards were fed. Early in this period individual hay allowances were adjusted according to live weight, or live weight estimated from chest girth measurements using the tables of Davis, Morgan, Brody & Ragsdale (15) or the equations given by Burt (16), when no weighing facilities were available (Exps. 1, 4 and 5). Concentrates were fed in accordance with individual production. Basal concentrate allocations for the experimental period were made in accordance with individual yields, or the mean yield of each block (Exp. 1 only), in the last week of the control period and were then adjusted according to the prescribed treatments. The basal concentrate allowance was reduced at the end of each experimental period by a standard amount (Exps. 1–3), or percentage (Exps. 4, 5), using the equalized feeding method of Lucas (17). Individual hay rations remained constant throughout each experiment, and a standard quantity of silage was fed.

The nutritive value of the foods used was estimated as follows:

Concentrates. By calculation using the values given by Woodman (2) for the constituent foods.

Roughages. Digestible nutrients were calculated from the proximate composition using appropriate digestibility coefficients from Schneider (18), and the protein equivalents of the hay and silage from the crude protein percentage of the dry matter using the equations given by Watson (19).

Starch equivalents were calculated from estimated digestible nutrients as follows:

Hay, S.E. = [digestible protein % × 0·94 + digestible ether extract % × 1·91 + digestible crude fibre % + digestible N.F.E. % − crude fibre % × 0·58] + $\frac{1}{5}$ of the value in square brackets (2, 20).

Silage, S.E. = digestible crude protein % × 0·94 + digestible ether extract % × 1·91 + digestible crude fibre % + digestible N.F.E. % − crude fibre % × 0·29 at 4% crude fibre in fresh material to 0·58 at 16% crude fibre (21).

The constituents of the concentrate mixtures used are shown in Table 1.

Table 1. *Constituents of the concentrate mixtures (parts by weight)*

Exp. 1	Exps. 2 and 3	Exps. 4 and 5
5 ground oats	5 ground oats	4 ground oats
2 grain balancer cubes (S.E. – 56·8, P.E. – 19·5)	3 ground barley	1 flaked maize
1 decorticated groundnut cake	1 decorticated groundnut cake	1 decorticated groundnut cake
1 linseed cake	1 linseed cake	1 linseed cake
Plus 1 lb. minerals and 2 lb. fish meal/cwt.	Plus 3 lb. minerals and 2 lb. fish meal/cwt.	Plus 1 lb. minerals/cwt.

Milk yields were recorded at each milking. Samples for the determination of fat and solids-not-fat percentage were taken each week in Exps. 1–3, and in the last week of each experimental period in Exps. 4–5. The samples were taken at four consecutive milkings, and individual bulks prepared for analysis in proportion to the respective yields. Fat percentages were determined by the Gerber method, total solids gravimetrically, and solids-not-fat by difference. The cows were weighed on one day each week and on two

consecutive days at the end of each experimental period in Exps. 2 and 3. Chest girths were recorded at the end of the control and each experimental period in Exps. 1, 4 and 5.

The results from the last week of each experimental period were analysed. The results of Exps. 2–5 inclusive were subjected to statistical analysis using the methods for change-over trials described by Patterson (22,23).

Experiment 1

This experiment was designed to provide preliminary data on the effects of feeding concentrates providing 75–150% of the normal production standards to cows in early lactation, and was carried out in early 1954.

The three treatment sequences used were:

Sequence	Period I	Period II
A (normal feeding)	3½ lb. concentrates/10 lb. milk after the first 15 lb	
B	As in A, plus concentrates equivalent to 25% of production	As in A, plus concentrates equivalent to 50% of production
C	As in A, minus concentrates equivalent to 25% of production	As in A

A basal ration of 15 lb. hay and 30 lb. grass silage was fed daily for maintenance and the production of 15 lb. milk by a cow weighing 1000 lb.

RESULTS

Mean daily yield during the experimental and preliminary and final control periods is shown for each treatment in Fig. 1, and the mean yields in the last week of each period in Table 2. One cow, formerly on treatment A, died during the first week of the final central period, and her yields for this, and the following 3 weeks were computed by the missing plot technique.

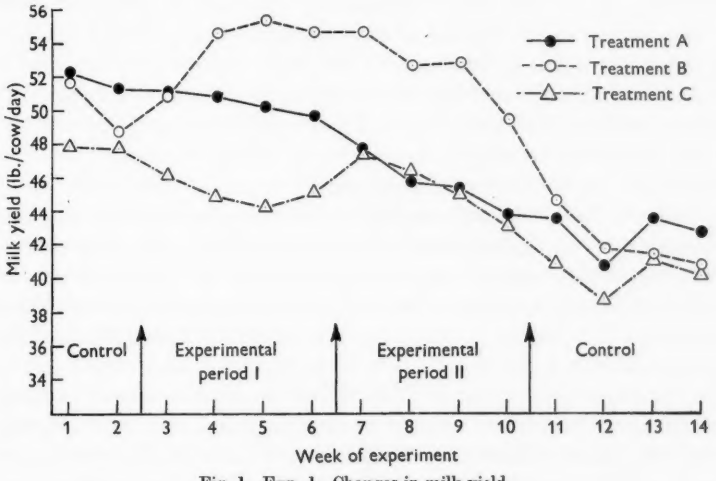

Fig. 1. Exp. 1. Changes in milk yield.

Average milk yield during the experiment was high, ranging from 4 to 5 gal. daily. In period I mean milk yield rose rapidly on treatment B, and fell rapidly on treatment C compared with the controls. At the end of this period mean daily yield was 5·0 lb. higher on treatment B, and 4·6 lb. lower on treatment C, than on treatment A. In period II, mean yield on treatment C rapidly recovered to a level similar to that on treatment A when similar amounts of concentrates were fed. The further addition of concentrates in treatment B had practically no effect on milk yield which was 5·6 lb./day higher than on

Table 2. *Exp.* 1. *Mean effects of treatment upon milk yield and chest girth*

	Milk yield (lb./day) in the last week of		Mean change in chest girth (in.) in	
Treatment	Period I	Period II	Period I	Period II
A	49·7	43.9	0·0	−0·5
B	54·7	49·5	+0·3	−0·2
C	45·1	43·1	−0·8	−0·7
Standard error	±2·8	±3·4	±0·3	±0·2

treatment A at the end of the period. The yield on treatment B dropped to the level of that on the other two treatments within 3 weeks after the end of period II. There was some divergence in the mean yields of the three groups of cows during the preliminary control period, due, at least in part, to the effects of disease, since one cow subsequently on treatment B and two destined for treatment C had attacks of acetonaemia. Analysis of variance showed that the effects of treatment upon milk yield were not statistically significant in either experimental period. The high experimental error (error standard deviation = 13% of the mean yield in period I) led to the use of changeover designs in subsequent experiments. Covariance analysis was of little benefit because of the effects of disease upon milk yields in the control period.

Changes in fat and solids-not-fat percentages are shown in Fig. 2. There were no effects of treatment on fat percentage, but solids-not-fat percentage declined more rapidly on treatment C than on A or B. However, this difference was not significant at the end of period I, and there was no evidence of any recovery when a normal ration was subsequently fed to the cows on treatment C. The mean changes in chest girth (Table 2) indicated that cows on treatment B maintained their live weight better than those on the other two treatments in both periods, but the differences were not significant.

There were practically no refusals of concentrates during this experiment. The refusals of roughage dry matter were greatest on treatment B in both periods, but the mean amount refused (1·4 lb./cow/day) was very small in comparison with the total dry matter fed which reached on average approximately 37 lb./day on this treatment in period II. The intakes of calculated production S.E. and P.E. (total intakes minus theoretical maintenance requirements) are shown in Table 3. Over 0·5 lb. P.E./10 lb. milk was fed on all treatments except treatment C in period I. S.E. intakes were approximately 85% of the normal standard for treatment A and 96 and 116% for treatment B, in periods I and II, respectively. The considerable divergence of these values from those originally planned was due to overestimation of the nutritive value of the roughages used, and the use of a farm-mixed concentrate which had not been properly balanced. It must also be remembered that the percentage levels of feeding on treatment B were reduced by the responses in milk yield

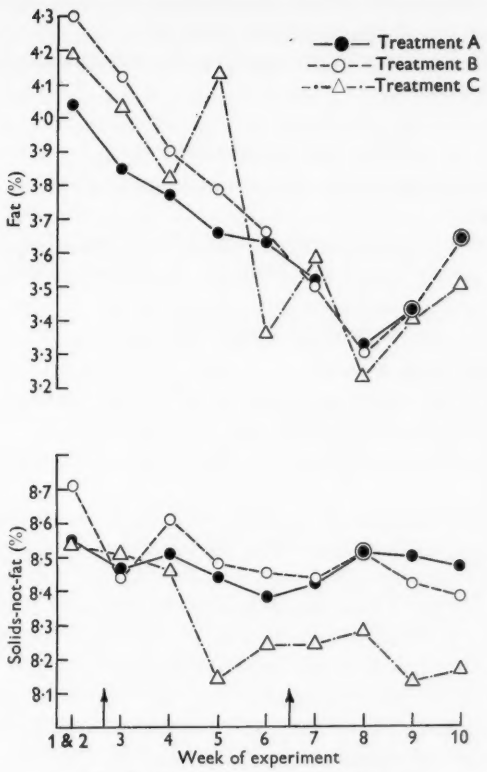

Fig. 2. Changes in milk composition.

Table 3. *Exp.* 1. *Calculated intakes of production starch equivalent and protein equivalent/10 lb. milk*

Treatment	lb. production S.E./10 lb. milk		lb. production P.E./10 lb. milk	
	Period I	Period II	Period I	Period II
A	2·11	2·16	0·53	0·54
B	2·41	2·89	0·61	0·73
C	1·69	2·19	0·42	0·54

which occurred. The results of this experiment indicated the need for more adequate control of error in further experiments. They indicated also that milk yields appeared to respond to increases in S.E. intake up to levels slightly in excess of the normal standards, and that high-yielding Ayrshire cows could consume up to 3·7 lb. dry matter/day/100 lb. live weight without digestive disturbance.

Experiments 2 *and* 3

These experiments were designed to study:

(*a*) The effects of feeding concentrates additional to the normal production standards in early and mid-lactation.

(*b*) The difference between the effects of additional high-carbohydrate food and those of additional balanced concentrates in early lactation.

(*c*) The effects of a limited substitution of hay for concentrates in mid-lactation.

The treatments used were:

Experiment 2 (*early lactation*)

(A) Normal feeding; roughage for maintenance and 10 lb. milk; $3\frac{3}{4}$ lb. concentrates/10 lb. milk thereafter.

(B) As in A, plus concentrates equivalent to 25% of production.

(C) As in A, plus ground barley supplying S.E. equivalent to 25% of production.

Experiment 3 (*mid-lactation*)

(A) Normal feeding; as in A above.

(B) As in A, plus concentrates equivalent to 25% of production.

(C) As in A, minus $1\frac{1}{2}$ lb. concentrates, plus 4 lb. hay daily.

The additional hay fed in treatment C of Exp. 3 was given in place of the afternoon feed of concentrates.

Because of the different stages of lactation required in the two experiments, and the nature of the distribution of calvings within the herd, it was not possible to meet all the requirements of either experiment simultaneously. The first four blocks of Exp. 2 and the first two blocks of Exp. 3 were therefore started in October 1954, and the remainder of each experiment in late December 1954. The average time from calving at the start of each experiment was 62 days in Exp. 2, and 139 days in Exp. 3.

A daily ration of 30 lb. grass silage and 15 lb. hay was fed to provide for the maintenance of a cow weighing 1000 lb. and the production of 10 lb. milk. The basal concentrate allocations of individual cows were reduced by 1 lb./day at the end of each experimental period in the first part of each experiment, and by $1\frac{1}{2}$ lb./day during the second part.

RESULTS

Experiment 2. The effects of treatment upon mean milk yield, fat and solids-not-fat percentages and live weight are shown in Table 4. One cow had acetonaemia during the last week of the first experimental period while on treatment A, and her data for this period were therefore calculated using the missing plot technique.

Table 4. *Exp.* 2. *Effects of treatment upon mean milk yield, fat and solids-not-fat percentages*

Treatment	Milk yield (lb./cow/day)	Fat (%)	Solids-not-fat (%)	Live weight (lb.)
A	39·9	3·63	8·28	1126·9
B	42·5	3·67	8·32	1130·8
C	41·4	3·70	8·33	1135·3
Standard error	±0·4	±0·07	±0·03	±4·4

Treatments B and C both produced significant responses in milk yield over the control, but the response to ground barley (C) was appreciably less than that to concentrates (B), although the difference was not quite statistically significant. Mean solids-not-fat

percentage and live weight were slightly higher for treatments B and C than for treatment A, but these differences were not statistically significant. There were no significant effects of treatment upon milk fat percentage. Mean refusals of roughage dry matter (Table 5), although relatively small, were appreciably higher for treatments B and C than for treatment A. Analysis of variance showed that the difference was significant and that there were also significant differences between cows. No relationship could be detected between individual roughage refusals and stage of lactation or milk yield. The increased refusal of roughage in treatments B and C was equivalent to 10% of the S.E. in the additional concentrates and barley fed on these treatments.

Table 5. *Exp.* 2. *Mean refusals of food, and intakes of production starch equivalent and protein equivalent/10 lb. milk*

Treatment	Refusals of roughage dry matter (lb./cow/day)	lb./10 lb. milk	
		Production S.E.	Production P.E.
A	0·8	2·48	0·55
B	1·6	2·91	0·64
C	1·6	3·02	0·59

The amounts of production S.E. consumed/10 lb. milk ranged from 2·48 to 3·02 lb. (Table 5) while P.E. intakes were above 0·5/10 lb. milk on all treatments.

Experiment 3. Mean milk yield, fat and solids-not-fat percentages, and live weight are shown for each treatment in Table 6. One cow suffered from atony of the rumen during the last week of the first experimental period, while another cow had a severe infection of one teat orifice which interfered with the withdrawal of milk in the last experimental period. Data for these cows and periods were calculated by the missing plot technique.

Mean milk yield in this experiment was 20–25 lb. daily as compared with over 40 lb. in Exp. 2. There was a significant response in milk yield (+1·3 lb./day) to the additional concentrates fed in treatment B, while the substitution of hay for concentrates in treatment C had no appreciable effect. There were no significant effects of treatment upon milk composition or live weight, but mean solids-not-fat percentage and live weight were slightly higher for treatment B than for treatments A and C.

Table 6. *Exp.* 3. *Effects of treatment upon mean milk yield, fat and solids-not-fat percentages and live weight*

Treatment	Milk yield (lb./cow/day)	Fat (%)	Solids-not-fat (%)	Live weight (lb.)
A	23·8	3·92	8·39	1079·9
B	25·1	3·84	8·44	1086·7
C	23·3	3·83	8·39	1077·4
Standard error	±0·3	±0·04	±0·03	±3·3

The average amounts of dry matter refused (Table 7) were highest on treatment C and lowest on treatment A. Dry matter refusals were significantly greater on treatment C than on the other two treatments, while the difference between treatments A and B was almost significant. There were significant differences between individual rates of refusal which could not be related to milk yield or stage of lactation. The additional roughage refused on treatment B was equivalent in S.E. value to approximately 10% of the

Table 7. *Exp.* 3. *Mean refusals of food and intakes of production starch equivalent and protein equivalent/10 lb. milk produced*

Treatment	Refusals of roughage dry matter (lb./cow/day)	lb./10 lb. milk	
		Production S.E.	Production P.E.
A	0·8	2·34	0·54
B	1·2	2·79	0·63
C	1·9	2·42	0·53

additional concentrates fed, while the refusal on treatment C merely adjusted the estimated S.E. in the additional hay to an amount equivalent to the reduction in the concentrate ration. The amounts of S.E. consumed above maintenance/10 lb. milk (Table 7) show that amounts slightly below the standard requirements were consumed on treatments A and C.

Experiments 4 *and* 5

The object of these experiments was to determine the effect of feeding production rations supplying 95, 115 and 135% of the normal production standards upon the productivity of Dairy Shorthorn and Friesian cows.

Experiment 4.

Material and methods. This experiment was carried out during early 1956 using eighteen Dairy Shorthorn cows and heifers. A basal ration of 40 lb. grass silage, 12 lb. hay and 1 lb. concentrates was fed for maintenance and the production of 10 lb. milk by a cow weighing 1120 lb. The concentrate mixture used provided 100% of normal production requirements when fed at $3\frac{3}{4}$ lb./10 lb. milk. The treatments used were: A, roughage and concentrates for maintenance and 95% of production S.E. requirements; B, roughage and concentrates for maintenance and 115% of production S.E. requirements; C, roughage and concentrates for maintenance and 135% of production S.E. requirements. All treatment differences were applied by varying the amount of concentrates fed.

Table 8. *Exp.* 4. *Effects of treatment upon mean milk yield, fat and solids-not-fat percentages and chest girth*

Treatment	Milk yield (lb./cow/day)	Fat (%)	Solids-not-fat (%)	Chest girth (in.)
A	21·9	3·60	8·59	75·4
B	22·9	3·51	8·62	75·3
C	23·2	3·66	8·71	75·6
Standard error	±0·5	+0·04	±0·02	±0·07

Results. Table 8 shows the effects of treatment upon mean milk yield, fat and solids-not-fat percentages and chest girth. Mean milk yield rose progressively with increasing level of feeding from 21·9 lb. on treatment A to 23·2 lb./day on treatment C, but this difference was not quite statistically significant. Mean fat percentage was significantly lower on treatment B than on treatment C, and there was a gradual increase in solids-not-fat percentage with increasing concentrate intake, the difference between the mean values for treatments A and C being significant. Mean chest girth was slightly greater for treatment C than for treatments A and B, but the difference was not significant.

The mean amounts of hay and silage refused were very small and there were no significant differences between treatments. There were, however, significant differences in individual rates of dry matter refusal which were unrelated to milk yield and stage of lactation. The actual average production S.E. and P.E. intakes (Table 9) were appreciably higher than those originally planned. This was due to an underestimate of the nutritive value of the roughages used, and to a relatively rapid decline in milk yield throughout the trial which was not adequately compensated by the changes in feeding made at each changeover.

Table 9. *Exp.* 4. *Mean intakes of production starch equivalent and protein equivalent/*10 *lb. milk*

Treatment	lb./10 lb. milk	
	Production S.E.	Production P.E.
A	2·67	0·77
B	3·17	0·87
C	3·79	1·01

Experiment 5.

Material and methods. This experiment was carried out in early 1956 using eighteen Friesian cows and heifers. A basal ration of 40 lb. grass silage and 15 lb. hay was fed for maintenance and the production of 10 lb. milk by a cow weighing 1200–1250 lb. The cows were housed in conventional cowsheds and milked in a milking parlour. Four pounds/day of a commercial dairy cube were fed in the milking parlour. The concentrate mixture used for production above 20 lb./day and to apply the treatment differences was similar to that used in Exp. 4 and the same treatments were compared.

Results. The mean effects of treatment upon milk yield, milk fat and solids-not-fat percentages and chest girth are shown in Table 10. There were significant treatment effects upon milk yields and solids-not-fat percentage, but not upon fat percentage or chest girth. The first incremental amount of concentrates (A–B) produced a significant increase of 1·3 lb. milk/cow/day, while the second incremental amount produced 0·7 lb./day which was not significant. Mean solids-not-fat percentage was significantly higher for treatment C than for treatments A and B. Mean chest girth was slightly higher at the higher levels of feeding, but the difference was not significant.

Table 10. *Exp.* 5. *Effects of treatment upon mean milk yield, fat and solids-not-fat percentages and chest girth*

Treatment	Milk yield (lb./cow/day)	Fat (%)	Solids-not-fat (%)	Chest girth (in.)
A	27·2	3·40	8·26	77·4
B	28·5	3·48	8·26	77·5
C	29·2	3·39	8·34	77·5
Standard error	±0·3	±0·05	±0·02	±0·13

The mean amounts of roughage dry matter refused (Table 11) were high, due to the hay used being of low palatability. There were no appreciable effects of treatment upon refusals, but there were again significant differences between individual cows which were not related to stage of lactation and milk yield. The mean production S.E. intakes (Table 11) were slightly lower than those originally planned due to the refusals of hay, but P.E. intakes were above the theoretical standards on all treatments.

Table 11. *Exp.* 5. *Mean refusals of food and intakes of production starch equivalent and protein equivalent*/10 lb. milk

Treatment	Refusals of roughage dry matter (lb./cow/day)	lb./10 lb. milk	
		Production S.E.	Production P.E.
A	2·6	2·19	0·60
B	2·2	2·71	0·71
C	2·2	3·24	0·83

DISCUSSION

Mean milk yield on all treatments involving balanced concentrate mixtures are plotted against the mean daily intakes of production S.E. in Fig. 3, which also contains a line describing the conventional feeding standard of 2·5 lb. S.E./10 lb. milk. It is clear that the responses (lb. milk/lb. additional S.E.) were appreciably less than the feeding standard ratio in all experiments, and that variations between the initial productivity of the different groups of cows used in these experiments had far more potent effects upon milk yield than did the amounts of concentrates fed.

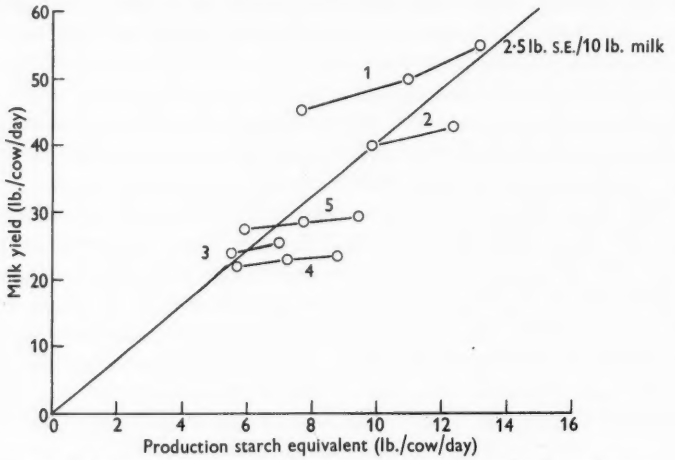

Fig. 3. Relationship between production starch equivalent and milk production.

When the responses in the different experiments were compared (Table 12), there was an indication that the response increased with milk yield (although the high errors and relatively low levels of feeding in Exp. 1 should be borne in mind). The regression of

Table 12. *Summary of mean responses in milk yield*

Exp.	Treatment comparison	Response (lb. milk/ lb. additional S.E.)	Mean control yield (treatment A; lb./day)
1	C–A	1·6	49·7
(Period I only)	A–B	1·9	—
2	A–B	1·0	39·9
3	A–B	0·9	23·8
4	A–B	0·7	21·9
	B–C	0·2	—
5	A–B	0·7	27·2
	B–C	0·4	—

response in yield (lb./lb. additional S.E.) between the treatments A and B upon mean yield (lb./day) on treatment A was calculated using data from individual blocks of three cows in Exps. 2, 3 and 5. Exp. 4 was omitted from this calculation because it showed no significant effects of treatment upon milk yield, and blocks of heifers were omitted as there were some indications, particularly in a later experiment (9), that heifers tend to respond less than cows. The regression coefficient of response upon mean milk yield was $+0{\cdot}03 \pm 0{\cdot}06$, which was not statistically significant, but is in good agreement with the value obtained in the later study of individual responses (9).

The variations in the level of feeding (lb. S.E./10 lb. milk) in the control treatments in three experiments were apparently unrelated to responses. A similar regression to the one already described of response upon mean control intake of production S.E./10 lb. milk was not significant and was in fact positive. However, in Exps. 4 and 5 where three levels of feeding were compared there were definite indications that the response to the second 20% addition to the production ration was approximately one-half of the response to the first additional 20%.

Comparison of responses in Exps. 2 and 3 gives no evidence of any appreciable effect of stage of lactation upon response, at least in the period from peak yield to 200 days. This finding is in agreement with the study of individual responses reported later (9).

The results of Exp. 2 suggest that feeding additional S.E. without the normal balance of P.E. may reduce responses in milk yield. This is interesting in the light of the recent work of Holmes and his collaborators (7), which has confirmed that amounts of digestible crude protein ranging from 0·46 to 0·66 lb./10 lb. milk have no effects upon production, but who found in a later experiment (8) that the use of a low protein concentrate mixture tended to limit the response of milk yield to additional S.E.

The limited substitution of hay for concentrates in Exp. 3 had no appreciable effect upon milk production when the calculated S.E. intakes at the two rates of hay feeding were similar. This suggests that the substitution values of hay and concentrates for milk production are adequately described by their respective starch equivalents, provided that adequate intakes of protein and other nutrients are maintained. This is in agreement with other experimental evidence (24,25,26) and with the conclusions of Jawetz (4), who considered that American reports of changing substitution values were due to the well-known overestimation of the energy value of roughage relative to concentrates when total digestible nutrients were used as the basis for substitution, and that, providing the energy values of the foods were properly evaluated, no change in substitution values could be detected within the range of normal feeding practice.

In Exps. 4 and 5, solids-not-fat percentages were increased at the higher levels of feeding, in agreement with the previous reports that marked underfeeding reduces solids-not-fat percentages (27,28) and that responses can occur at levels of feeding above the normal standards (7,29). However, the absence of any appreciable responses in Exps. 2 and 3, where mean percentages were well below 8·5% emphasizes that low values can and often do occur in well managed herds fed adequate winter rations, due to genetic effects or possibly to unknown nutritional factors, and that this condition may not be appreciably affected by increasing food intake.

The significant differences between mean roughage refusals by individual cows on Exps. 2–5 indicate that widespread variations in individual appetite for roughage occur, even when the amount of roughage offered is adjusted to allow for individual differences

in live weight, and is not excessive in amount. The significance of such variations was investigated in a later experiment (9).

Mean responses in milk yield, illustrated in Table 12, amounted to 0·7–1·0 lb. milk/lb. additional S.E. in Exps. 2–5 inclusive. Wholesale milk prices for winter milk in 1955–56 averaged 4·4*d.*/lb. (Southern region, plus T.T. Attested bonus, minus transport changes), while the cost of the concentrate mixtures used amounted to 5*d.*–6*d.*/lb. S.E. during the same period. At the mean price of 5·5*d.*/lb. S.E. the minimum economic response in milk yield (i.e. that at which additional food cost and the return from additional output are equal) is 1·25 lb. milk/lb. S.E. This value is greater than the responses obtained in these experiments, and the additional feeding was therefore not generally economic at prices ruling in 1955–56.

When considering these results it must be remembered that the experiments were conducted during the period between peak yield and about the 200th day of lactation upon cows which had been steamed up and fed adequate rations during the period before peak yield. The responses to additional feeding in the period before peak yield have been subjected to very little experimental study and further work is needed, while the response to concentrates in the steaming-up ration is greater than that reported here for the declining phase of lactation (6).

SUMMARY

1. A series of five experiments on the effects of variation in the concentrate ration of cows receiving normal diets of hay and grass silage are described.

2. Responses in milk yield to the feeding of additional concentrates occurred in all five experiments and were statistically significant in three of them.

3. Mean responses in four experiments with adequate statistical control ranged from 0·7 to 1·0 lb. milk/lb. additional S.E. at levels of feeding from 90 to 120% of the Woodman standard, and were not economic at prices ruling during the winter of 1955–56.

4. There were indications that the responses varied with the yield of the cow, and in two experiments there was clear evidence that the response to a second addition of 20% in the production ration was approximately half of that to the first.

5. The response to additional S.E. was appreciably reduced when normal protein standards were not maintained in the additional concentrates.

6. A limited substitution of hay for concentrates, which did not alter S.E. intakes appreciably, had no appreciable effect on milk yield.

7. Significant responses of solids-not-fat percentage to the feeding of concentrates above the normal standards occurred in two experiments, but not in two others where the control percentages were lower. The importance of factors other than energy intake in influencing this quantity was emphasized.

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636.2.084:612.664.31

678. THE EFFECT OF VARIATIONS IN NUTRIENT INTAKE UPON THE YIELD AND COMPOSITION OF MILK

II. FACTORS AFFECTING RATE OF EATING ROUGHAGE AND RESPONSES TO AN INCREASE IN THE AMOUNT OF CONCENTRATES FED

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(With 5 Figures)

The practical application of the results of closely controlled experiments on the effects of different levels of concentrate intake may be affected by the lack of control of roughage intakes of individual cows which occurs in most dairy herds. Individual cows show considerable variation in their ability to ingest and utilize roughage (1,2,3), which, together with the effects of competition, can give rise to considerable differences in individual plane of nutrition when roughages are rationed on a 'herd' basis. Responses to additional concentrates might therefore be expected to be maximal in those cows which are on the lower planes of nutrition due to low roughage intakes. The experiment to be described was therefore designed in an attempt to assess the importance of the effects of variations in individual rates of eating roughage upon responses to a standard increase in the concentrate ration under a system of feeding and management commonly used in dairy herds in the South of England.

MATERIAL AND METHODS

Twenty-nine Ayrshire cattle were used. They were divided into two groups (fourteen cows and fifteen heifers) and were housed in separate but adjacent covered yards fitted with mangers for individual feeding. The animals were given a daily 3 hr. exercise period on a bare paddock and were milked in a milking parlour. No concentrates were fed in the milking parlour. The daily feeding routine was as follows: 4.15 a.m., half of the daily allowance of concentrates; 5.0 a.m., milking; 6.30–7.30 a.m., half the daily allowance of hay; 9.45–11.0 a.m., marrowstem kale; 11.0 a.m.–2.0 p.m., exercise; 2.45 p.m., the other half of the concentrates; 4.0 p.m., milking; 5.0–6.0 p.m. the other half of the hay.

The animals had access to water at all times except when tied in the yokes for feeding.

Table 1. *Mean age, stage of lactation and milk yield at the start of the experiment*

	Age (months)	Stage of lactation (days from calving)	Milk yield (lb./week)
	Yard 1 (15 heifers)		
Mean	33·2	54	226
S.D.	3·8	9	31
	Yard 2 (14 cows)		
Mean	81·4	119	278
S.D.	23·3	61	69

The mean age, stage of lactation, and milk yield in each yard and their respective standard deviations are shown in Table 1, which shows that the cows were a more variable group than the heifers.

The plan of the experiment was as follows:

Period	Duration (weeks)	Treatment
I	2	Measurement of rates of eating roughage. Concentrates for all milk after the first 10 lb./cow/day. Individual rates of eating measured
II	2	Yard feeding—control period. Concentrates as in period I. Roughages fed as a total amount per yard based on average intakes during period I
III	3	Yard feeding—increased concentrates. Roughage as in period II, all concentrate allowances increased by 3 lb./day
IV	2	Final control period and remeasurement of rates of eating. All concentrate allocations as in period III but reduced by 4 lb./day. Individual rates of eating determined.

Foods. The chemical composition and estimated nutritive value of the foods used are shown in Table 2. Sufficient hay from one field was not available to last throughout the trial, and material of a slightly lower protein content was fed during period IV. The slight change in composition of the marrowstem kale between periods I and IV may be attributed to the increasing maturity of the crop. The concentrate mixture consisted of 4 parts ground oats, 4 parts ground barley, 1 part decorticated groundnut cake, 2 parts linseed cake, $\frac{1}{8}$ part fish meal and 1 lb./cwt. of a commercial mineral mixture with $1\frac{1}{2}$ lb./cwt. calcined magnesite. The mixture was fed at the rate of $3\frac{3}{4}$ lb./10 lb. milk.

Table 2. *Proximate composition and estimated nutritive value of the foods used*

		Percentage as fed							Estimated nutritive value	
Food	Period	Moisture	Crude protein	True protein	Ash	Ether extract	Crude fibre	Nitrogen-free extract	Starch equivalent	Protein equivalent
Marrowstem kale	I	87·9	2·0	1·4	1·6	0·2	1·9	6·3	7·3	1·3
	IV	86·2	1·8	1·3	1·6	0·2	2·3	7·9	8·8	1·2
Hay	I	19·7	8·8	7·9	6·5	1·2	28·3	35·5	36·0	4·4
	IV	21·8	7·0	6·0	5·7	1·0	28·4	36·1	35·0	3·2
Concentrates		9·8	21·2	20·7	5·8	4·3	6·9	52·0	65·5	13·7

Determination of rates of eating. Hay was offered at two daily feeds of 60 min. duration, and kale at one daily feed of 60 min. Initially, weighed standard quantities were offered, and supplementary weighed amounts were added to individual rations as soon as the smallness of the residues seemed likely to limit consumption. At the end of each 60 min. period the residues were removed, weighed, and subsequently fed to other stock. All weights were recorded to the nearest $\frac{1}{4}$ lb. Determinations of rates of eating at the morning and afternoon hay feeds and the kale feed were made on eleven occasions during period I (the first of which was omitted from subsequent analysis) and on ten occasions during period IV. At feeds during these periods when it was not desired to determine rates of eating the cows were secured at the same time and fed as much as they would consume in 60 min.

Rates of eating concentrates were measured at the afternoon concentrate feed on four occasions during each of periods I and IV. Only one yard was studied on any one day.

The cows were fed a weighed standard amount of the mixture practically simultaneously, and individual consumption times recorded with a stopwatch to the nearest second. These determinations were not completely objective, since the precise moment of cessation of eating is rather difficult to define when meal mixtures are fed. The end point used occurred when practically no meal was left and the cow began to clean her manger. The standard amounts offered were 5 and 4 lb. to the cows, and 4 and 3 lb. to the heifers, in periods I and IV, respectively. The remainder of the individual daily concentrate rations were fed at the following morning feed.

Kale dry-matter percentages

Samples for the determination of the dry-matter percentage of the kale fed were taken on as many days as possible during periods I and IV. As there were no facilities for carrying out dry-matter determinations on the farm, these samples were sent by post to Shinfield. The samples were weighed immediately after sampling, and again immediately before the determination of dry-matter percentage, and a correction was made for the moisture lost in transit.

Other observations

Milk samples for the determination of fat and solids-not-fat percentages were taken in each week of the experiment after week 1. Live weights were recorded on three successive days at the end of each period of the experiment after the morning hay feed. Measurements of chest and paunch girths were recorded on two consecutive days at the end of period II. Meteorological observations were recorded at a station approximately 300 yards from the site of the experiment.

Observations were made on several occasions in an attempt to determine the social or 'bunting' order in each yard by recording the outcome of 'contests' between cows (4). On two days during periods II and III the number of cows in each yard engaged in various activities (lying, standing, ruminating, eating, drinking, moving and cleaning) was recorded at 15 min. intervals for a period of 24 hr.

RESULTS

The results for each yard were treated separately in practically all the analyses, since the cows and heifers were distinct populations in respect of age and variability (Table 1).

Changes in milk yield, milk quality and liveweight

Mean values in each week of the experiment are shown for each yard in Fig. 1. The changes which occurred were very similar in the two yards. Mean milk yield declined steadily during the experiment, apart from period III when the extra concentrates fed checked the decline. Very little change in live weight occurred although there was a slight loss in period III (approximately 12 lb.) which was subsequently regained. Fat percentage declined slightly and solids-not-fat percentage rose slightly during period III.

Measurements of rates of eating

Kale. The variation in mean rates of eating, summarized in Table 3, shows that there were considerable differences between individual mean rates of eating and between daily mean rates in both yards and periods. Analysis of variance (for example see Tables 4

and 5) showed that these individual and daily differences were significant, that mean rate of eating increased significantly in both yards between periods I and IV, and that there were significant cow × period interactions. Rate of eating increased between periods I

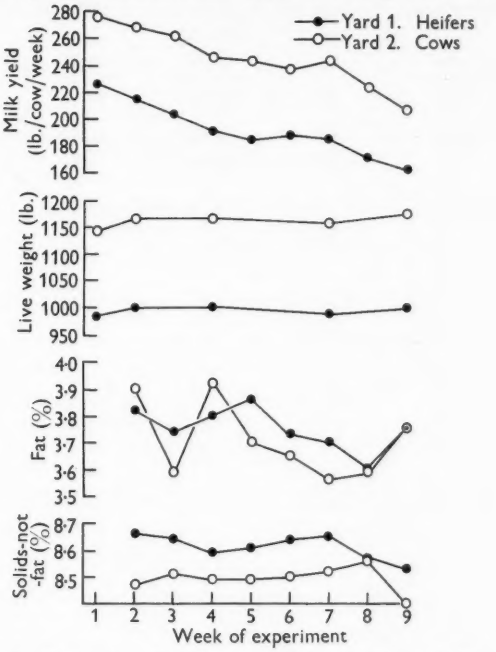

Fig. 1. Changes in milk yield, live weight and milk composition.

Table 3. *Summary of mean rates of eating fresh kale (lb./60 min.)*

		Individual mean rates of eating			Mean daily rates of eating				
Yard	Period	Minimum	Maximum	S.D.	Minimum	Maximum	S.D.	Period mean	Overall mean
1 (heifers)	I	23·9	30·4	1·74	23·9	33·4	3·07	27·4	28·6
	IV	24·7	34·4	2·65	27·5	31·7	1·15	29·7	
2 (cows)	I	24·9	38·5	3·75	28·4	34·6	2·02	31·0	32·4
	IV	29·9	39·5	3·41	30·8	35·8	1·76	33·7	

Table 4. *Analysis of variance of rate of eating fresh kale (lb./60 min.). Yard* 1 *(heifers), period I*

Factor	D.F.	S.S.	M.S.	*F*
Total	149	2479·2175	—	—
Between cows	14	421·5113	30·11	4·8**
Between days	9	1268·02	140·89	22·5**
Error	126	0789·6862	6·27	—

Statistical significance is indicated thus: ** Highly significant ($P<0·01$); * Significant ($P>0·01$ and $<0·05$); N.S. Not significant ($P>0·05$).

and IV by 2–3 lb./60 min., and was consistently 3·5–4·0 lb./60 min. greater in the cows than in the heifers. The standard deviations show that individual variation was more important than day to day variation apart from the results for the heifers in period I.

Kale dry-matter percentages were determined on 8 days during period I, and 6 days

Table 5. *Analysis of variance of rate of eating fresh kale (lb./60 min.). Yard 1 (heifers) periods I and IV*

Factor	D.F.	S.S.	M.S.	*F*
Total	299	4890·687	—	—
Between cows	14	813·297	58·09	9·0**
Between periods	1	405·422	405·42	62·6**
Between days within periods	18	1446·699	80·37	12·4**
Cows × periods	14	593·300	42·38	6·5**
Error	252	1631·969	6·48	—

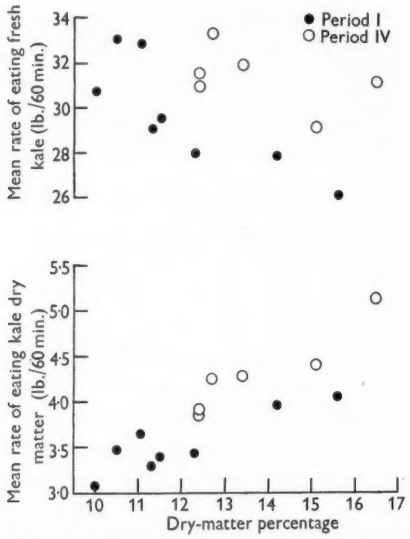

Fig. 2. Effect of dry-matter percentage upon rate of eating kale.

during period IV. The average daily rates of eating fresh kale and kale dry matter on these occasions are plotted against dry-matter percentages in Fig. 2. Although the rate of eating fresh kale tended to decrease with increasing dry-matter percentage, there was a marked linear association between dry-matter percentage and rate of consumption of dry matter within both periods, the regressions of mean rate of eating (lb./60 min.) on dry-matter percentage being $+0{\cdot}155 \pm 0{\cdot}032$ and $+0{\cdot}254 \pm 0{\cdot}048$ in periods I and IV, respectively. Approximately 80% of the variation in mean daily rates of eating dry matter was accounted for by the variations in dry-matter percentage.

Hay. The mean rates of eating hay are summarized in Table 6. The differences between cows and between days were significant in both yards and periods (e.g. see analyses of variance in Tables 7 and 8). The standard deviations show that variation between individuals was much more important than that between days, and was greater in the cows than in the heifers. Rates of eating were generally slightly higher at the afternoon than at the morning feed, and this difference was significant except in yard 2 in period IV. The cows consistently ate 1·5–2·0 lb./60 min. more than the heifers. Mean rates of eating increased by 1 lb./60 min. in the heifers and 0·5 lb. in the cows between periods I and IV. This increase was significant in both yards, as were the cow × period interactions.

Table 6. *Summary of mean rates of eating hay*

		Individual mean rates of eating (lb./120 min.)				Total (lb./120 min.)			Mean daily rates of eating: Morning (lb./60 min.)			Mean daily rates of eating: Afternoon (lb./60 min)		
Yard	Period	Minimum	Maximum	Mean	S.D.	Minimum	Maximum	S.D.	Minimum	Maximum	Mean	Minimum	Maximum	Mean
1 (heifers)	I	9·6	14·7	11·5	1·61	10·6	13·1	0·77	5·0	6·2	5·6	5·5	6·9	5·9
	IV	11·7	16·7	13·5	1·23	12·6	14·3	0·49	6·2	7·3	6·6	6·3	7·3	6·9
2 (Cows)	I	11·9	19·2	15·3	2·09	14·3	16·1	0·66	6·8	7·9	7·5	6·9	8·4	7·8
	IV	13·9	19·4	16·3	1·97	16·0	16·6	0·19	7·9	8·4	8·1	8·0	8·3	8·2

Table 7. *Analysis of variance of rate of eating hay (lb./60 min.). Yard 1 (Heifers), period I*

Factor	D.F.	S.S.	M.S.	*F*
Total	299	440·2665	—	—
Between cows	14	181·0696	12·93	20·0**
Between days	9	38·743	4·31	6·6**
Morning v. afternoon	1	22·006	22·01	4·0**
Cows × days	126	87·4227	0·69	1·07 N.S.
Cows × morning v. afternoon	14	20·9846	1·50	2·31**
Days × morning v. afternoon	9	8·3342	0·93	1·43 N.S.
Error	126	81·7064	0·65	—

Table 8. *Analysis of variance of rate of eating hay (lb./120 min.). Yard 1 (Heifers) periods I and IV*

Factor	D.F.	S.S	M.S.	*F*
Total	299	1493·8023	—	—
Between cows	14	395·0304	28·22	16·3**
Between periods	1	152·7352	152·7	88·5**
Days within periods	18	219·9671	12·22	7·08**
Cows × periods	14	290·9054	20·78	12·0**
Error	252	435·1642	1·73	—

Concentrates. The results were expressed as consumption times (i.e. seconds taken to consume 1 lb.) and are summarized in Table 9. The differences between individuals and between days were significant in both yards and periods, as were the cow × period interactions. Mean consumption times increased slightly between periods I and IV, but

Table 9. *Summary of mean concentrate consumption times (seconds/lb.)*

		Individual means				Daily means		
Yard	Period	Minimum	Maximum	Mean	S.D.	Minimum	Maximum	S.D.
1 (heifers)	I	120	187	151	17·8	138	168	14·3
	IV	124	180	153	14·8	151	156	2·3
2 (cows)	I	88	149	115	16·1	105	120	6·8
	IV	92	149	118	17·7	110	126	6·3

the differences were not significant. The cows ate appreciably faster than the heifers, the mean consumption times/lb. being approximately 2 and 2½ min., respectively. Again, the standard deviations show that individual variation was more important than day to day differences and that the cows were more variable than the heifers. Correlation analysis showed that there was generally a small and non-significant negative association between consumption time and the total daily ration of concentrates, i.e. those cows generally

receiving more concentrates ate faster. However, this relationship is inextricably confounded with that between appetite and milk yield discussed below, since the amount of concentrates normally fed varied with milk yield.

Errors in the determination of rates of eating. The effect of repeated observations upon the errors associated with mean rates of eating are shown in Fig. 3, which includes coefficients of variation of mean rates of eating calculated from observations on 1–10 days. The results show that coefficients of variation fell rapidly as the number of observations increased from 1 to 3, 4 or 5, but that further increase in the number of observations had relatively little effect upon error. The scheme of observation used in this study, therefore, satisfactorily minimized the errors associated with individual mean rates of eating.

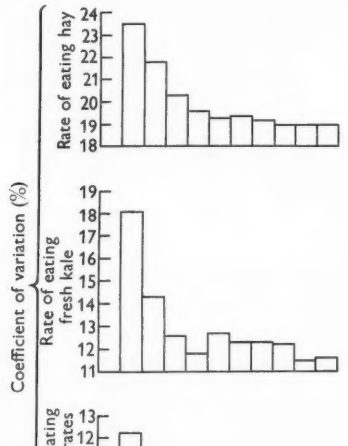

Fig. 3. Effect of repeated observations upon variation in mean rates of eating.

Estimates of the components of variance associated with error (σ^2) and between days (σ_D^2) were calculated from the analyses of variance of rates of eating by the usual methods (5). For fresh kale the standard error of observations made on the same day was $\pm 2{\cdot}59$ lb./60 min. compared with $\pm 3{\cdot}29$ for observations made on random days. Taking observations on the same day, therefore, reduced errors by 21%, while doubling the number of observations reduced errors by 30%. Similar standard errors for mean rates of eating hay/day (lb./120 min) were $\pm 1{\cdot}19$ and $\pm 1{\cdot}36$ for contemporary and random observations, respectively, and those for concentrates $\pm 9{\cdot}6$ and $\pm 11{\cdot}3$ sec./lb., respectively.

The more marked effects of day-to-day errors in rate of eating fresh kale may be attributed to the effects of variations in dry-matter percentage described above.

Factors affecting rate of eating

The relationship between individual rates of eating of the three foods (Table 10). These relationships were positive in all cases (negative correlations with concentrate consumption times indicate positive relationships with rates of eating), and were most marked for hay

Table 10. *The correlations of individual rates of eating hay and fresh kale and concentrate consumption times*

Yard	Period	Variables: Concentrate consumption time and rate of eating hay	Concentrate consumption time and rate of eating fresh kale	Rates of eating fresh kale and hay
1 (heifers)	I	−0·051 N.S.	−0·034 N.S.	+0·664**
	IV	−0·008 N.S.	−0·599*	+0·321 N.S.
2 (cows)	I	−0·560*	−0·499 N.S.	+0·755**
	IV	−0·208 N.S.	−0·416 N.S.	+0·606*

Table 11. *The correlations of live weight with rates of eating hay and kale and concentrate consumption times*

Yard	Period	Correlation of live weight with: Rates of eating: Hay	Rates of eating: Kale	Concentrate consumption time
1 (heifers)	I	+0·439 N.S.	+0·100 N.S.	−0·391 N.S.
	IV	+0·432 N.S.	−0·177 N.S.	+0·096 N.S.
2 (cows)	I	+0·622*	+0·497 N.S.	−0·372 N.S.
	IV	+0·589*	+0·209 N.S.	−0·145 N.S.

Regressions of rate of eating hay (lb./120 min.) upon live weight (lb.) in yard 2: period I, $Y = 0{\cdot}0145X - 1{\cdot}71$; period II, $Y = 0{\cdot}0125X + 1{\cdot}81$.

and kale where three of the four correlation coefficients were significant in spite of the small number of degrees of freedom (12 or 13). The coefficients were generally greater in the cows than in the heifers, which probably reflects the greater variation in individual rates of eating among the cows.

Live weight. The correlations between live weight and individual rates of eating (Table 11) show that in practically all cases the association was positive. The correlations tended to be greater for hay than the other foods, and more marked in the cows than in the heifers. The regression equations at the bottom of the table show that the daily consumption of hay (lb./120 min.) tended to increase by 1·2–1·5 lb. for each 100 lb. increase in live weight in the cows. The plotted data gave no indication of curvilinearity.

Body conformation. The correlations between mean rates of eating roughage and body measurements are shown in Table 12. None of these were significant in the heifers, but those for the cows were all positive and were significant for rate of eating hay with both measurements. The multiple regression of rate of eating hay (lb./120 min.) (Y) upon chest girth (X_1) and paunch girth (X_2) (both in in.) was

$$Y = 0{\cdot}675X_1 + 0{\cdot}124X_2 - 44{\cdot}9$$

and the values of 't' for the respective partial regression coefficients were 3·063* and 1·131 (N.S.).

Table 12. *Correlations of rates of eating roughage with body measurements*

Yard	Rate of eating hay with: Chest girth	Rate of eating hay with: Paunch girth	Rate of eating kale with: Chest girth	Rate of eating kale with: Paunch girth
1 (heifers)	+0·361 N.S.	+0·257 N.S.	−0·109 N.S.	−0·456 N.S.
2 (cows)	+0·808**	+0·572*	+0·171 N.S.	+0·440 N.S.

Table 13. *The correlations of milk yield with rate of eating roughages*

Yard	Period	Correlations between milk yield and rate of eating: Hay	Kale
1 (heifers)	I	+0·143 N.S.	+0·134 N.S.
	IV	+0·100 N.S.	−0·156 N.S.
2 (cows)	I	+0·211 N.S.	+0·102 N.S.
	IV	+0·126 N.S.	−0·132 N.S.

Table 14. *Correlations between individual rates of eating in periods I and IV*

Yard	Hay (afternoon)	Hay (morning)	Total hay	Fresh kale	Concentrates
1 (heifers)	+0·375 N.S.	+0·339 N.S.	+0·385 N.S.	+0·172 N.S.	+0·673**
2 (cows)	+0·941**	+0·989**	+0·909**	+0·767**	+0·743**

Milk yield and stage of lactation. The correlations of milk yield with rates of eating hay and kale (Table 13) were positive in most cases, but were small and not significant. No relationship could be detected between stage of lactation and rates of eating, or with the individual changes in the latter between periods I and IV.

Cow × period interactions. The combined analyses of variance of rates of eating in periods I and IV showed that significant cow × period interactions occurred in both yards for all three foods, i.e. the rank order of individual rates of eating changed significantly during the experiment. The importance of these interactions is inversely related to the degree of positive association between individual mean rates of eating in periods I and IV (Table 14). These correlations show that cow × period interactions were relatively more important in the heifers than in the cows, and were least important for rates of eating hay in the cows where the correlations were greater than +0·9. The correlations in Table 15, although not significant in most cases, show that there was a general tendency for changes in individual appetite between periods I and IV to be reflected in the rates of eating of all three foods.

Table 15. *Correlations between the changes in rate of eating of the different foods within individuals between periods I and IV*

Yard	Hay and fresh kale	Hay and concentrate consumption time	Fresh kale and concentrate consumption time
1 (heifers)	+0·509 N.S.	−0·210 N.S.	−0·037 N.S.
2 (cows)	+0·477 N.S.	−0·479 N.S.	−0·669**

Table 16. *Correlations between rates of eating recorded simultaneously in the two yards*

Period	Hay (afternoon)	Hay (morning)	Total hay	Fresh kale
I	+0·640*	+0·691*	+0·754*	+0·807**
IV	−0·038 N.S.	+0·656*	+0·207 N.S.	+0·928**

Climate. The correlations in Table 16 show that an appreciable proportion of the day-to-day variation in rates of eating was generally common to both yards, although the notably high correlations for fresh kale were undoubtedly influenced by the effects of dry-matter percentage already described. The relationships between rates of eating and

climatic conditions were examined in detail(6), but no clear relationships were found. However, there was an indication that lower minimum temperatures were associated with higher rates of eating the morning ration of hay in period IV when the mean minimum temperature was 35° F, but not in period I, when it was 7° F higher.

The relationship between rates of eating at adjacent feeds. These correlations were practically all small, positive and not significant, showing that the relationships were affected by external factors extending over more than one feed, rather than any tendency for a high rate of eating at one feed to be followed by a low rate of eating at the next.

The effects of variations in rate of eating upon nutrient intake relative to requirements

Individual mean rates of eating in periods I and IV were used to compute mean daily nutrient intakes from roughage. Mean starch equivalent intakes relative to individual maintenance requirements are summarized in Table 17. In both periods, roughage consumption in the time allowed provided average starch equivalent (S.E.) intakes in excess of

Table 17. *Comparison of roughage nutrient intakes with maintenance requirements*

		Roughage S.E. minus maintenance S.E.		Roughage S.E. as % of maintenance S.E.	
Yard	Period	Mean (lb./day)	S.D.	Mean	S.D.
1 (heifers)	I	+0·24	0·63	104	10·6
	IV	+1·37	0·58	123	10·5
2 (cows)	I	+1·00	0·80	115	11·8
	IV	+1·90	0·80	129	11·4

maintenance requirements, and the excess was significantly greater in the cows than in the heifers. The standard deviations again reflect the considerable individual variation in rates of eating roughage, and these rates had marked effects on the plane of nutrition of individual cows since the correlations between (roughage S.E. minus maintenance requirements) and (total S.E. minus total requirements) ranged from +0·70 to +0·83 and were all significant. The rise in rates of eating between periods I and IV is reflected in the greater excess of mean S.E. intakes over maintenance requirements in the latter period.

The effects of the increase in the concentrate ration

Individual responses to the additional 3 lb. of concentrates/day fed during period III were calculated as follows:

$$\text{Response} = X_3 - \frac{X_2 + X_4}{2},$$

where X_2, X_3 and X_4 are the appropriate quantities (e.g. milk yield) in the last week of periods II, III and IV, respectively. Mean responses in each yard and their standard errors are shown in Table 18. The mean increase in milk yield and reduction in live weight were statistically significant in both yards, but the decrease in fat percentage was significant only in the cows, and the increase in solids-not-fat percentage only in the heifers. The mean effects upon solids-not-fat percentage and live weight were very similar in the two yards, but the increase in milk yield and decline in fat percentage were more marked in the cows than in the heifers. The difference between the yard means was

statistically significant for fat percentage, but not for milk yield. The correlations between individual responses in the four variables are shown for each yard in Table 19. Only one of these, the positive correlation between responses in milk yield and solids-not-fat percentage in the cows, was significant. However, there were indications that high responses in solids-not-fat percentage tended to be associated with high responses in milk yield and low live-weight loss.

Table 18. *Mean responses to the additional concentrates fed in period III*

	Response in							
	Milk yield (lb./week)		Fat (%)		Solids-not-fat (%)		Live weight (lb.)	
Yard	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error
1 (heifers)	+ 8·0	±3·0	−0·02	±0·08	+0·09	±0·02	−11·2	±2·4
2 (cows)	+14·3	±2·4	−0·27	±0·07	+0·08	±0·05	−14·4	±4·4

Table 19. *Correlations between individual responses in milk yield, fat and solids-not-fat percentages and live weight in period III*

Yard	Milk yield and live weight	Milk yield and fat %	Milk yield and solids-not-fat (%)	Fat % and live weight	Solids-not-fat % and live weight	Fat and solids-not fat percentages
1 (heifers)	−0·143 N.S.	−0·160 N.S.	+0·318 N.S.	+0·091 N.S.	+0·328 N.S.	−0·036 N.S.
2 (cows)	−0·104 N.S.	+0·425 N.S.	+0·545*	−0·466 N.S.	+0·254 N.S.	+0·024 N.S.

Table 20. *The relation of response in milk yield (period III) with milk yield and with stage of lactation*

	Response in milk yield (lb./week) and	
Yard	Milk yield (lb./week)	Stage of lactation (days)
	Correlation coefficients	
1 (heifers)	+0·352 N.S.	−0·059 N.S.
2 (cows)	+0·583*	−0·687**
	Regression coefficients	
2 (cows)	+0·069	−0·032

Progressive analysis of variance of multiple regression of response in milk yield (yard 2)

	D.F.		S.S.		
Independent variate	Regression	Residual	Residual	Last variate	F (last variate)
—	—	13	1030·4257	—	—
Milk yield (X_1)	1	12	680·4725	349·9532	6·17*
Milk yield (X_1) and stage of lactation (X_2)	2	11	637·6599	42·8126	N.S.

$$y = 0{\cdot}089x_1 + 0{\cdot}039x_2.$$

The correlations of the response in milk yield with milk yield (mean of the last weeks of periods II and IV) and with stage of lactation are shown in Table 20. In both cases the correlations were positive with milk yield and negative with stage of lactation, and were statistically significant for the cows but not for the heifers. Multiple regression analysis (Table 20) showed that there was no relationship between response and stage of lactation independent of milk yield, and no tendency whatsoever for response to diminish with advance in stage of lactation when account had been taken of milk yield.

The regression equation of response in milk yield (lb./week) (Y) upon milk yield (lb./week) (X) was

$$Y = 0{\cdot}069X - 1{\cdot}5.$$

This shows that a 100 lb. difference in weekly yield was associated with a difference of 6·9 lb./week in the response to an extra 3 lb. of concentrates/day. Examination of the results for the cows showed that responses ranged from +2 lb./week at a yield of 104 lb. to +34 lb./week at a yield of 384 lb., and no curvilinearity could be detected when the data were plotted.

The mean yield of the heifers in yard 1 was 177 lb./week which was well within the range of yields in yard 2. The predicted response at this yield using the above regression was 10·7 lb./week which was appreciably greater than the mean observed response of 8·0 lb./week.

Table 21. *Regression analysis of solids-not-fat responses* (Y) *in the cows* (*Yard* 2)

Independent variate	Residual D.F.	Residual S.S.	S.S. (last variate)	F (last variate)
—	13	0·3900	—	—
Mean S.N.F. (X_1)	12	0·2488	0·1412	6·81*
Mean S.N.F. (X_1) and mean milk yield (X_2)	11	0·2414	0·0074	N.S.
Mean S.N.F. (X_1) and response in milk yield (X_3)	11	0·2030	0·0458	2·48 N.S.
Mean S.N.F. (X_1), X_3 and response in live weight (X_4)	10	0·1537	0·0493	3·21 N.S.

Regression equations: $Y = 2{\cdot}669 - 0{\cdot}307\,(X_1)$;

$Y = 0{\cdot}0076\,X_3 + 0{\cdot}00375\,X_4 - 0{\cdot}2541\,X_1 - 2{\cdot}013$;

$t = 1{\cdot}8105$ N.S. $1{\cdot}7936$ N.S. $2{\cdot}306$*.

The standard errors in Table 18 indicated that variation in individual responses in solids-not-fat percentage was much greater in the cows than in the heifers. A multiple regression analysis of factors which might influence these responses was therefore carried out (Table 21). The regression of response in solids-not-fat percentage (Y) upon mean solids-not-fat percentage in the last weeks of periods II and IV was $-0{\cdot}307 \pm 0{\cdot}118$, which was statistically significant and showed that response tended to decrease at higher basal solids-not-fat percentages. The addition of mean milk yield as a second variate had no appreciable effect, but the successive addition of response in milk yield (X_3) and response in live weight (X_4) produced F values which were appreciable, as were the values of t for their regression coefficients in the equation at the bottom of the table. However, only the coefficient for solids-not-fat percentage was significant. The results of the whole analysis show that there was a tendency for the response in solids-not-fat percentage to increase with increasing responses in milk yield and live weight, and that it was significantly lower in those cows with high percentages in periods II and IV.

There was no significant association between response in live weight and mean live weight at the end of periods II and IV.

The relationship between individual rates of eating and the response to additional concentrates

The rank order of individual rates of eating roughage changed significantly in both yards between periods I and IV as shown by the cow × period interactions already described. Since the responses to the additional concentrates were determined using the

data from the last week of period III which immediately preceded the redetermination of rates of eating roughage in period IV, the latter were correlated with individual responses in milk yield, solids-not-fat percentage and live weight with the results shown in Table 22. There were no appreciable relationships between responses and rates of eating roughage in the heifers. In the cows there was no appreciable relationship between rates of eating

Table 22. *Correlations of individual rates of eating roughages in period IV with responses to the additional concentrates fed in period III*

	Response in milk yield and		Response in solids-not-fat percentage and		Response in live weight and	
Yard	Rate of eating hay	Rate of eating kale	Rate of eating hay	Rate of eating kale	Rate of eating hay	Rate of eating kale
1 (heifers)	+0·210 N.S.	−0·070 N.S.	−0·195 N.S.	+0·037 N.S.	−0·241 N.S.	−0·054 N.S.
2 (cows)	−0·067 N.S.	+0·062 N.S.	−0·376 N.S.	−0·139 N.S.	−0·687**	−0·708**

and responses in milk yield, but the correlations with responses in solids-not-fat were negative but not significant, and those with responses in live weight negative and significant. Since rate of eating hay had already been shown to be positively correlated with live weight in the cows, and significant relationships between response in milk yield and milk yield, and response in solids-not-fat and solids-not-fat percentage, had also been demonstrated, multiple regression analysis was carried out. The multiple regressions of response in yield upon milk yield, rate of eating hay and live weight; of response in solids-not-fat percentage upon solids-not-fat percentage, rate of eating hay and live weight; and of response in live weight upon rate of eating hay and live weight were calculated, but in no case did the partial regression coefficient of response upon rate of eating hay differ appreciably in size or significance from the simple regression coefficients. The values of the latter were:

Response in milk yield (lb./week) upon rate of eating hay (lb./120 min.) $-0{\cdot}301 \pm 1{\cdot}30$.
Response in solids-not-fat (%) upon rate of eating hay (lb./120 min.) $-0{\cdot}033 \pm 0{\cdot}020$.
Response in live weight (lb.) upon rate of eating hay (lb./120 min.) $-5{\cdot}78 \pm 1{\cdot}77$.

Animal behaviour

The results of contests between individual cows in each yard (4), described in detail elsewhere (6), were not sufficiently numerous to establish more than a provisional 'bunting' order for most of the cows. There was no sign of any diminution in response in yield to additional concentrates at higher levels in the 'bunting order', but there were indications that rates of eating and live weights were higher at the higher levels in the 'bunting' order. General observation showed that practically no obvious competition for food occurred in periods II and III when the animals were group fed, in fact most of the contests were observed at times when some factor operated to cause a general movement among the cows.

The percentage of cows lying down and the percentage ruminating, recorded at 15 min. intervals during a 24 hr. period, are shown in Figs. 4 and 5 for the cows and heifers, respectively. These values were obtained by averaging the results for the 2 days of observation, and the results were very similar in the two yards. After 09.00 the percentage of animals lying and ruminating declined with the approach of the 09.45 feed of

Percentage ruminating
Percentage lying
Percentage ruminating
Percentage lying

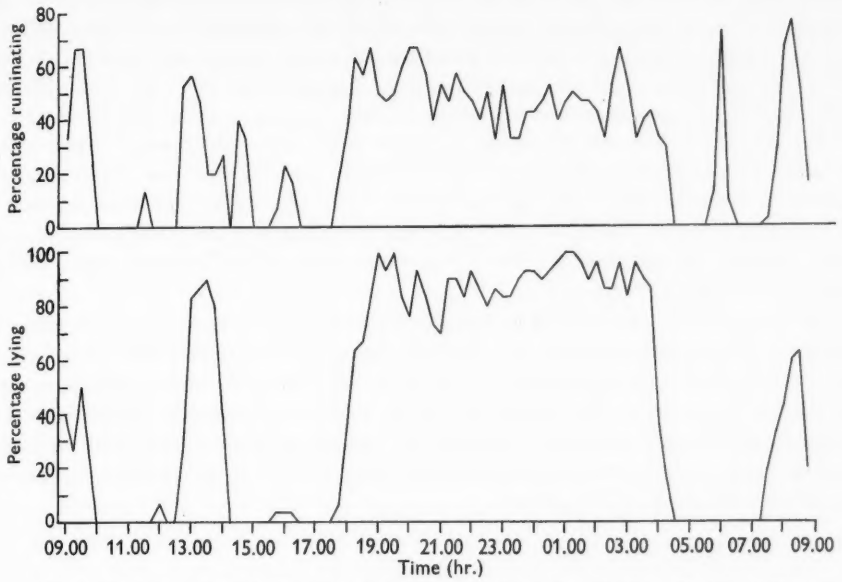

Fig. 4. Diurnal variation in the percentage of animals lying and ruminating. Yard 1. Heifers.

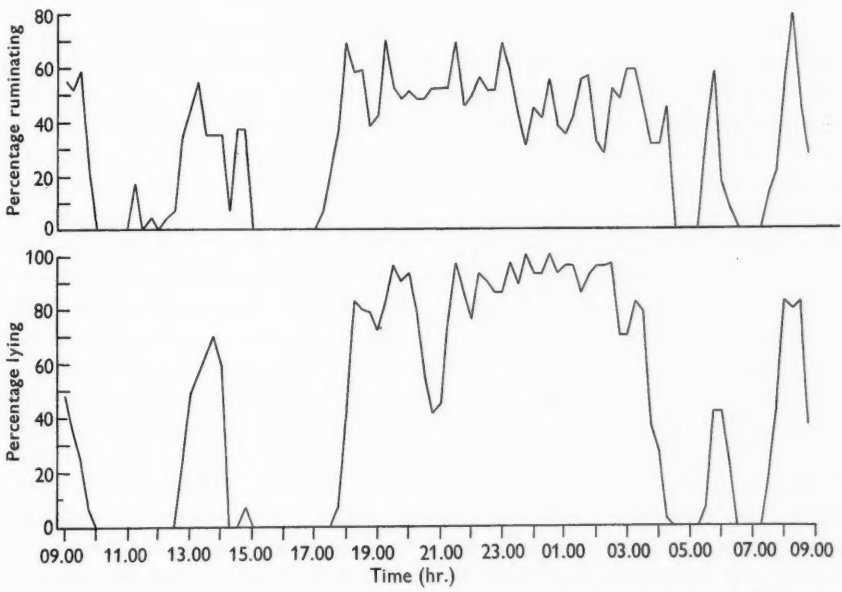

Fig. 5. Diurnal variation in the percentage of animals lying and ruminating. Yard 2. Cows.

kale. When the cattle had been turned out to bare paddocks at 11.15 lying down and rumination became increasingly evident after 30–60 min. Eating concentrates, milking, and the second hay feed of the day occupied the afternoon, but the cattle quickly settled down after the lights in the yards were turned out at approximately 17.30. From 18.00 to 03.30 the following morning 80–90% of the animals were lying down and 40–50% were ruminating at any time. The percentage lying tended to rise slightly, and the percentage ruminating to decline slightly as the night advanced. At 03.30 the cows began to get up and move about in anticipation of the arrival of a herdsman and the morning feed of concentrates at 04.15. After this feed and the morning milking there was a further short period of rest and rumination before the morning feed of hay at 06.15, and a similar period from 07.30 to 08.45.

The average time out of the 24 hr. spent lying-down and ruminating in each yard was computed (mean percentage at the ninety-six observations × 24 hr.). The average lying time was 10 hr. 50 min. in the heifers and 10 hr. 35 min. in the cows, and the average rumination times were 7 hr. 18 min. and 7 hr. 32 min., respectively. Observations at 15 min. intervals did not provide a picture of drinking and cleaning activities worthy of detailed analysis, but general observation indicated that most of the water intake was drunk after the feeding of roughages.

DISCUSSION

One of the most striking features of the results of this experiment is the highly significant variation in individual rates of eating which occurred in both yards and periods for all three foods, and which confirms the observations of previous workers(1,2,3,7). There were positive correlations between the rates of eating of the three foods by the same cow, indicating that a common appetite factor was affecting the reactions of the individual to the different foods, and there were important associations between live weight and rate of eating, particularly of hay. In the cows the rate of eating hay (lb./120 min.) increased by 1·35 lb./100 lb. increase in live weight. The difference in mean live weight between the cows and heifers was 160 lb., giving a predicted difference of 2·2 lb. in rate of eating hay compared with the actual mean difference of 3·3 lb. This shows that cows ate relatively faster than heifers of the same live weight, but the difference may have been affected by stage of lactation which averaged 54 days from calving at the start of the experiment in the heifers and 119 days in the cows, since Graves, Dawson, Kopland, Watt & Van Horn(8) found that the average consumption of lucerne hay given *ad lib.* increased appreciably during the first 5 months of lactation. However, in the present study, no effect of stage of lactation upon rates of eating could be detected in either yard or period, nor was it related to individual changes in rates of eating between periods I and IV.

The correlations between live weight and rates of eating showed that, at most, only 35–40% of variation in rates of eating were accounted for by differences in live weight, and the corresponding variations in roughage nutrient intake relative to maintenance requirements were considerable. For instance in the cows in period IV the amount of roughage S.E. consumed in addition to theoretical maintenance requirements ranged from 0·75 to 3·42 lb. daily.

The suggestion, in the cow data, of a positive relationship between rate of eating hay and paunch girth independent of body size indicates that there may be some truth in the

commonly accepted association between development in the abdominal region and the ability to ingest and utilize large quantities of roughage.

In contrast to the results obtained elsewhere under other systems of management, no appreciable relationship between milk yield and rate of eating was observed in this study. Kruger and his co-workers have found positive associations between dry-matter intake and milk yield (3) and similar observations on the grazing dairy cow have been made on many occasions (9), 10). A positive relationship between milk yield and grazing time was found by Hancock (11, 12, 13) but not by Waite, MacDonald & Holmes (14). However, these observations were all made on cows receiving no food in addition to grazing, whereas concentrates were fed at $3\frac{3}{4}$ lb./10 lb. milk after the first 10 lb. in the present experiment. The feeding of 1 lb. concentrates/5 lb. milk was found to reduce daily grazing time by 1 hr. in experiments in New Zealand (12, 13), while Jensen, Klein, Rauchenstein, Woodward & Smith (15) observed a 50–70 lb. decrease in the amount of hay eaten for each 100 lb. increase in the amount of concentrates fed when good roughage was offered *ad lib.* Individual differences in the amount of concentrates fed probably eliminated differences in appetite related to milk yield in the present study, and it is noteworthy that there was no indication of any negative association between milk yield and rate of eating concentrates, in spite of the feeding of increasing amounts of concentrates to the higher-yielding cows.

There were considerable and significant day-to-day variations in the mean rates of eating each food within each yard and period, and the correlations between mean rates of eating recorded in the two yards on the same day confirm that these were influenced by common factors affecting all the animals. Much of the variation in rate of eating fresh kale was associated with its dry-matter percentage, the rate of ingestion of dry matter rising by approximately 0·2 lb./60 min./1 % increase in dry-matter percentage. Similar relationships in silage feeding have been reported by others (16, 17). However, most of these observations and those of the present study are subject to an unknown effect due to error correlation, because of the positive association between errors in dry-matter percentage and dry-matter intake, when the same estimate of dry-matter percentage is used in both cases.

There was no evidence of any marked and consistent effect of climatic conditions upon rates of eating. The indications of a negative association between morning rates of eating and minimum temperature in period IV when the mean minimum temperature was less than 40° F agree with the psychroenergetic studies at Missouri (18), where food intakes rose at temperatures below 40° F, but did not change appreciably between 40 and 70° F.

Rates of eating at adjacent feeds were positively associated due to the effects of common factors upon appetite, and there was no indication of any tendency for a high rate of eating at one feed to be followed by a low rate of eating at the next. This is not surprising in view of the restricted feeding times used which prevented the satiation of appetite at any one feed.

The study of the errors involved in the estimation of individual rates of eating showed that these were estimated satisfactorily from observations recorded on 4–10 days and that day-to-day variations would markedly increase the error involved if observations recorded at different times were used to compare individuals.

Since it was not possible to carry out a comprehensive programme of sampling and chemical analysis of the foods used, minor day-to-day and animal-to-animal variations in composition may have contributed to the day-to-day variation in rates of eating and

to the errors. Blaxter & French (1) reported a positive association between rate of eating and the quality of hay from the same stack, while Woodward, Shepherd & Graves (19) found that differences in palatability between seven types of hay were associated with mean intakes ranging from 8–15 lb. daily when these were fed in conjunction with standard grain and silage rations. The practical effects of differences of this type in palatability seem worthy of further study because of their limiting effects upon the utilization of roughage.

The significant cow × period interactions in rate of eating showed that the relative rates of eating were not stable throughout the experiment, particularly in the heifers. Correlation analysis showed that these changes were related in part to individual changes in appetite for all foods. Individual changes in rate of eating were not associated with stage of lactation nor with individual changes in milk yield during the experiment. Rates of eating generally increased between periods I and IV, and this change may have been influenced by increasing adaptation to winter rations, by the advance in stage of lactation, or by the seasonal fall in temperature.

The animal behaviour results may be compared with observations made under other systems of management. Waite, MacDonald & Holmes (14) reported an average daily lying time of 10 hr. in a study of grazing dairy cows extending over one grazing season, while Johnstone-Wallace & Kennedy (20) reported average lying and rumination times of 12 and 7 hr., respectively for four beef cows. Fuller (21) found that cows tied in stanchions lay down for 50% of the time and ruminated for a total of 8–8½ hr. daily. Castle, Foot & Halley (22) reported average lying and rumination times of 9·2 and 5·6 hr. for four cows at grass, and also noted that only about 50% of cows housed at night in conventional cowsheds during the winter months were lying down at any one time, while on summer grazing 90% lay down by 1–1½ hr. after sunset. The results of the present study, which showed that 80–90% of cows housed in covered yards were lying at any one time during the night interval, tend to confirm the practical belief that cows are more comfortable in covered yards than in conventional cowsheds. However, no disturbance due to oestrus occurred while these observations were being made, and this can cause considerable upset among cows housed in a yard. The average lying and rumination times of 10½–11 hr. and 7–7½ hr., respectively, compare favourably with the observations quoted above.

The response to a standard amount of additional concentrates was significantly and positively related to milk yield, and there were no independent effects due to stage of lactation. These observations confirm the results of a previous analysis of a series of conventional feeding experiments (23), and the regressions contrast with the results of Jensen *et al.* (15) who reported that responses to increased feeding were very similar in 'good' and 'poor' producing cows when the comparison was made at the same percentage of the Haecker production standards for total digestible nutrients. The regression of response (lb. additional milk/lb. additional S.E.) upon milk yield (lb./day) was $+0{\cdot}035 \pm 0{\cdot}04$ which is in very close agreement with the value previously reported (23). The predicted response ranged from 0·6 lb. milk/lb. additional S.E. at a daily yield of 20 to 1·4 lb. at 40 lb., and the difference is obviously of economic importance, since the minimum economic response at prices ruling during the 1955–56 winter was approximately 1·25 lb. milk/lb. S.E., indicating that it would not be economic to feed 3 lb. of additional concentrates to cows giving less than 3½–4 gal./day.

It has been suggested (24) that the American practice of feeding roughage *ad lib.* with a standard amount of concentrates for all milk produced, should be adopted more widely in this country in order to encourage the maximum use of cheap roughage and to simplify herd management. However, this would probably result in the high-yielding cows being underfed relative to the remainder of the herd, and the existence of a positive relationship between milk yield and responses to additional feeding emphasize the danger of uneconomic distribution of food inherent in such a system.

The significant negative correlation between solids-not-fat percentage and the solids-not-fat response to additional concentrates showed that those cows having inherently low percentages responded most, but this relationship, unlike that between milk yield and the response in yield, is subject to an unknown degree of error correlation, since any error reducing recorded solids-not-fat percentages in periods II or IV automatically increased the measured response and vice versa.

There were no obvious relationships between rates of eating roughage and the responses to additional concentrates in the heifers. In the cows, responses in milk yield were unrelated to rates of eating, but negative correlations became progressively more important for responses in solids-not-fat percentage and live weight, and were significant for the latter. This indicates that the general level of feeding in this experiment was such that responses in yield were not limited by nutritional factors, but by the capacity of the mammary gland to react, and that variations in underlying nutritional level were therefore reflected in the responses in live weight. The absence of any relationship between rates of eating roughage and response in milk yield can reasonably be attributed to the general level of feeding used, since responses do not change very rapidly with alteration in level of feeding at levels round about the normal standards (15,25), and to the relatively high concentrate : roughage ratio of the diet which limited the expression of individual capacities for ingestion and utilization of roughage. It may be concluded, therefore, that when roughages are fed for maintenance and the first gallon and concentrates for remaining production, and when cows and heifers are separated to limit the effects of competition on roughage intake, individual appetite for roughage does not have any marked effect upon responses to additional concentrates. However, there remains the possibility, which should be investigated, that such effects might be important when diets containing a ratio of roughage : concentrates higher than that used in this experiment are fed.

Variations in individual appetite for roughage may be of considerable economic importance, since those cows which can consume and utilize relatively large quantities of roughage will demand correspondingly smaller amounts of expensive concentrates. However, the results of the present study and the work quoted in the discussion illustrate the difficulties which beset the adequate estimation of individual ability to consume roughage. Age, milk yield, stage of lactation, type and quality of the food, and the amount of concentrates fed may all have appreciable effects, together with less evident factors such as management and climatic conditions.

SUMMARY

1. Rates of eating hay, kale and concentrates and responses to a 3 lb. increase in the daily concentrate allowances when roughage was rationed on a 'group' basis, were measured in fourteen Ayrshire cows and fifteen heifers.

2. There were significant differences between animals, between days, and significant cow × period interactions, in rates of eating in both groups. The cows were a more variable group and generally ate faster than the heifers. Although the rate of eating fresh kale tended to decrease with increasing dry-matter percentage, the rate of eating kale dry matter was positively and significantly related to the dry-matter percentage.

3. There were positive associations between the rates of eating of the three different foods, and of these with live weight, but no effects of milk yield or stage of lactation, and no marked effects of climatic conditions could be detected.

4. The errors in the determination of mean rates of eating were such that observations made on 4–5 days gave satisfactory comparisons of individual rates of eating.

5. Reponses in milk yield to additional concentrates were positively correlated with milk yield, and no independent effects of stage of lactation could be detected. Responses by the heifers were less than those of cows giving similar yields.

6. Responses in solids-not-fat percentage were greatest in those cows with inherently low percentages.

7. There was no relationship between rates of eating roughage and responses of milk yield to additional concentrates, but live-weight responses and, to a lesser extent, solids-not-fat responses were negatively correlated with rates of eating in the cows. It was concluded that the level of feeding, system of management and the roughage:concentrate ratio used were such as to eliminate the effects of rates of eating roughage upon milk yields, if indeed such effects exist.

The author is greatly indebted to Mr W. D. Hay, Farm Director, Ministry of Agriculture, Fisheries and Food, Bridget's Experimental Husbandry Farm and to the farm staff for their collaboration in carrying out this experiment. Thanks are due also to Dr S. Bartlett and Dr S. J. Rowland for their help and advice, to Mrs M. C. Holdsworth, Mrs R. E. Winder and Mr A. W. Wagstaff who analysed the food and milk samples, and to Mr D. Zeremski who did much of the computation.

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679. THE RELATIONSHIP BETWEEN MILKING RATE AND MILK YIELD

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Introduction

Previous investigations have shown that 'within cows', the rate of machine milking is correlated with the milk yield at the recorded milking. This is so when the yield variation is due to milking intervals varying in length as well as to different stages in the lactation.

Especially when the difference in milk yield depends on the different lengths of the milking intervals at the same stage in the lactation, it is reasonable to suppose that the faster milking after the longer interval is due to a higher udder pressure, caused by the larger volume of milk. However, it has been shown by Dodd (1) that the decrease in milking rate during the lactation is not solely due to the change in milk volume.

New Zealand Dairy Board (2), Andreae (3) and Sandvik (4) have shown that the milk yield affects the 'between cows' variation in the milking rate too. Andreae, therefore, has suggested that the measurements of the milking rate be corrected for the correlation with the milk yield. A system of correction has been worked out by Sandvik (4) and an extract of this is given in Table 1.

Table 1. *Correction of the measurements of milking rate for the correlation with milk yield* (4)

kg. of milk at the recorded milking. Average for morning and evening	Addition to (+) or subtraction from (−) the average of morning and evening observations		
	Machine time	Peak flow	Percentage yield in 3 min.
4·5– 5·4	+0·64	+0·28	− 7·90
5·5– 6·4	+0·32	+0·14	− 3·90
6·5– 7·4	±0	±0	± 0
7·5– 8·4	−0·32	−0·14	+ 3·90
8·5– 9·4	−0·64	−0·28	+ 7·90
9·5–10·4	−0·96	−0·42	+11·80
10·5–11·4	−1·28	−0·55	+15·80
per kg.	−0·3223	−0·1386	+ 3·9411

The relationship between the average milking characteristics and milk yield in 300 days, maximum daily yield and persistency

The question whether slow-milking cows usually have larger amounts of residual milk left in their alveolar tissue at the end of the milking, thereby causing decreased production and persistency, is very interesting. The results of the previous investigations on this problem are not in full agreement. Dodd & Foot (5) reported that for each pound per minute increase in peak flow, the yield in 305 days increases by 419 lb. The relationship was significant at the 1% level. They also found a significant relationship between peak flow and maximum daily yield, lactation length, lactation solids-not-fat percentage and persistency. Brumby (6), however, did not find that maximum flow rate was related to peak yield or lactation yield.

In the opinion of the author the question cannot, however, be answered by correlating the cows' average milking characteristics with lactation yield and with persistency. In most cases the lactation yield is correlated with the milk yield at the time when the milking characters were recorded. It has also been shown by Johansson & Hansson (7) that persistency is correlated with lactation yield. Because of the correlation between the milking characteristics and the yield at the recorded milkings, significant correlation coefficients may be found even when slow milking does not necessarily lead to lower production.

To get an answer to this question, the lactation yield and the persistency must be compared with milking characteristics recorded when the milk yield was approximately the same for all the cows, or the observed milking characteristics may be corrected for the correlation to the milk yield at the recorded milkings.

In this investigation, which was carried out at the Institute of Animal Breeding and Genetics at the Agricultural College of Norway, both methods mentioned above have been used.

MATERIAL AND METHODS

The data used for the analyses presented were collected from 57 heifers of Norwegian Red Poll, and 111 heifers of Norwegian Red and White Cattle. The Red Polls were recorded at the Hafslund Progeny Testing Station. The recording of the Reds and Whites was carried out by field control in Hedmark county.

The heifers of the Reds and Whites had been sired by twelve bulls, and the Red Polls by four bulls used for artificial insemination.

The recording was done by continuous weighing of the milking machine bucket, and by weight readings at half-minute intervals. All cows were recorded at milking time in the morning as well as in the evening. At the Hafslund Progeny Testing Station the milking characteristics of the cows were recorded from four to twenty times during the first 180 days of lactation. In the field control the cows were recorded from four to eight times during the first 230 days of lactation. The milking characteristics used in the analyses are means of all recordings for the same cow. The milk yield as well as the composition of the milk was recorded one day every week at Hafslund, and one day in every 3 weeks in the field control. At Hafslund all cows were hand-stripped. In the field control hand-stripping was not practised. Definitions of some terms used in the analyses are given below:

Machine time (min.): duration of machine milking up to the start of the machine stripping. *Peak flow* (kg./min.): yield during the minute of maximum flow. *Percentage yield in 3 min.*: the milk yield during the first 3 min. as percentage of the total yield. *Standard lactation yield* (kg.): amount of milk produced in period up to 300 days, or until dry. *Maximum daily yield* (kg.): the highest recorded daily yield. *Persistency*: (*a*) at Hafslund. Number of days from calving till daily yield falls below one-half of maximum daily yield. (*b*) At Hedmark. Percentage yield in 100 days.

RESULTS

In Table 2 are presented the correlation and regression coefficients of the milking characteristics on milk yield and persistency.

As in the investigation by Dodd & Foot, highly significant coefficients were found between standard lactation yield and uncorrelated peak flow. In the field records the

Table 2. *The relationship between milking characteristics (independent variables) and milk yield and persistency (dependent variables)*

Independent variables ...	Observed machine time (min.)		Corrected machine time (min.)		Observed peak flow kg./min.		Corrected peak flow kg./min.		Observed percentage yield in 3 min.		Corrected percentage yield in 3 min.		No. of cows
Dependent variables	*r*	*b*	*r*	*b*	*r*	*b*	*r*	*b*	*r*	*b*	*r*	*b*	
Standard lactation yield													
All recordings at Hafslund	0·05	16	−0·20	−72	0·41**	391	0·14	141	−0·04	−1	0·21	6	57
Cows recorded at Hafslund when milk yield was 6·1–7·0 kg.	−0·22	−76	—	—	0·12	117	—	—	0·28	8	—	—	52
Field records at Hedmark	0·25***	134	0·11	6	0·32***	439	0·05	69	−0·72***	−1	0·05	1	111
Maximum daily yield													
All recordings at Hafslund	0·25	0·47	0·07	0·02	0·23	1·18	−0·05	−0·27	−0·23	−0·04	0·02	0·00	57
Cows recorded at Hafslund when milk yield was 6·1–7·0 kg.	−0·08	−0·16	—	—	0·04	0·25	—	—	0·14	0·02	—	—	52
Persistency													
All recordings at Hafslund[1]	−0·16	−4·2	−0·15	−4·2	0·14	9·9	0·16	12·2	0·14	0·3	0·13	0·3	57
Cows recorded at Hafslund when milk yield was 6·1–7·0 kg.[1]	−0·08	−2·2	—	—	0·06	4·6	—	—	0·08	0·2	—	—	52
Field records at Hedmark[2]	−0·08	−0·2	−0·06	−0·2	0·06	0·52	0·11	0·92	0·10	0·03	0·10	0·03	111

[1] No. of days after calving before the daily yield falls below one-half of the maximum daily yield.
[2] Percentage yield in 100 days.
* Significant at the 5% level. ** Significant at the 1% level. *** Significant at the 0·1% level.

correlation coefficients of observed machine time and observed percentage yield in 3 min. on standard lactation yield were significant also.

For cows at Hafslund tested when the milk yield was 6·1–7·0 kg., no significant coefficients were found. This was also the case when the milking characteristics were corrected for the effect of the milk yield at the recorded milkings.

Because the milk yield at Hedmark was recorded only every 3 weeks, the correlation between maximum daily yield and milking characteristics has been calculated for Hafslund only. For uncorrected peak flow the regression coefficient does not differ very much from that found by Dodd & Foot; it is, however, not significant. The correlation coefficients of observed machine time, and observed percentage yield in 3 min. on maximum daily yield, are of about the same size as those between observed peak flow and maximum daily yield. When the milking characteristics were corrected for the effect of the milk yield at the recorded milkings, or when the cows were recorded when the milk yield was between 6·1 and 7·0 kg., however, all correlations coefficients were close to zero.

The regression coefficient between uncorrected peak flow and persistency for all recordings at Hafslund, was nearly the same as that found by Dodd & Foot. No correlation coefficient between milking characteristics and persistency, however, was significant. Nor were the coefficients reduced by the correction for the effect of milk yield at the recorded milking. For the cows recorded when the milk yield was 6·1–7·0 kg., the coefficients were still lower, and of the same size as those from the field recordings, where the milk yield was recorded only once every 3 weeks, and the persistency was consequently stated as percentage yield in 100 days.

To the extent that this investigation is comparable with that by Dodd & Foot, the results are very similar. When the effect of milk yield at the recorded milking on the observed milking characteristics was eliminated, no significant correlation coefficients were found between milking characteristics, standard lactation yield, maximum daily yield, or persistency. This was so when the effect of the milk yield at the recorded milking was eliminated by correction as well as by using only those observations of milking characteristics that were taken when the milk yield was between 6·1 and 7·0 kg.

As mentioned already, when we wish to investigate to what extent lactation yield, maximum daily yield, and persistency are dependent on milking rate, the milking characteristics must be corrected for the effect of milk yield at the recorded milkings. The conclusion to be drawn from this investigation is, therefore, that the milking character has no significant effect on lactation yield or persistency. Slow-milking cows, therefore, need not have low milk production.

Summary

This paper deals with an investigation on the influence of milking characteristics on lactation yield, maximum daily yield, and persistency carried out at the Institute of Animal Breeding and Genetics at the Agricultural College of Norway.

Data used for the analyses were collected from 57 heifers of Norwegian Red Poll and 111 heifers of Norwegian Red and White Cattle. The Red Polls were recorded at the Hafslund Progeny Testing Station, while the recording of the Reds and Whites were carried out by field control.

When the effect of the milk yield at the recorded milkings on the observed milking

characteristics was eliminated, the milking character had no significant effect on the lactation yield, the maximum daily yield, or the persistency. This was the case when the effect of the milk yield at the recorded milkings was eliminated by correction as well as by using only those observations of milking characteristics that were taken when the milk yield was between 6·1 and 7·0 kg. Slow-milking cows are not, therefore, necessarily poor milk producers.

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680. SOME PROPERTIES OF THE MAMMARY MYOEPITHELIUM OF THE SOW

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(With 6 Figures)

In earlier studies (1,2) on the effects of oxytocin and other active materials on the pressure within the mammary gland of the sow during milk ejection, it became apparent that under some conditions instead of a simple rise followed by an immediate fall in pressure, a complex pressure curve could be produced. Cases were recorded in which two or three distinct waves of pressure occurred following the injection of quantities of oxytocin which were somewhat greater than required to produce a normal ejection. Such multiple curves were recorded only at levels of oxytocin such as would be associated with the release of more than a normal quantity of milk, and would therefore seem to be above physiological levels. The general shape of the multiple milk ejection pressure curve bore some resemblance to intrauterine pressure curves and this suggested that a more thorough investigation should be made into the nature of these multiple pressure curves with a view to making a quantitative comparison with intrauterine pressure changes. It seemed that it would be valuable to obtain information on the frequency characteristics and on the relationship between pressure curves obtained simultaneously from different glands.

METHODS

Two mammary glands situated as far apart as possible were selected for recording, and were cannulated using the simple teat siphon arrangement described earlier (3). The cannulae were connected to pressure recording heads which are described in the appendix. The outputs from the two heads were recorded independently on two strip-chart recorders run at identical speeds. The timing of injections was done by suddenly pinching both leads from the cannulae so that identical timing marks were made by the two recorders, thus making it possible to make precise comparisons between such variables as the interval between injection and the initial rise in pressure. All injections were made into an ear vein of the sow. Glands were chosen which were well stocked and which were readily cannulated. For this reason the first or second gland was not always chosen because of big differences in the development level of individual glands.

RESULTS

Fig. 1 sets out a series of double recordings showing the change in pressure within the sow mammary glands plotted against time. The upper graph represents the pectoral gland, the lower one the inguinal. All graphs are characterized by a sudden rise in pressure followed by a fall which is sometimes irregular. This is followed by a period of oscillation. It should be noted that the doses used to produce this oscillation would be regarded as

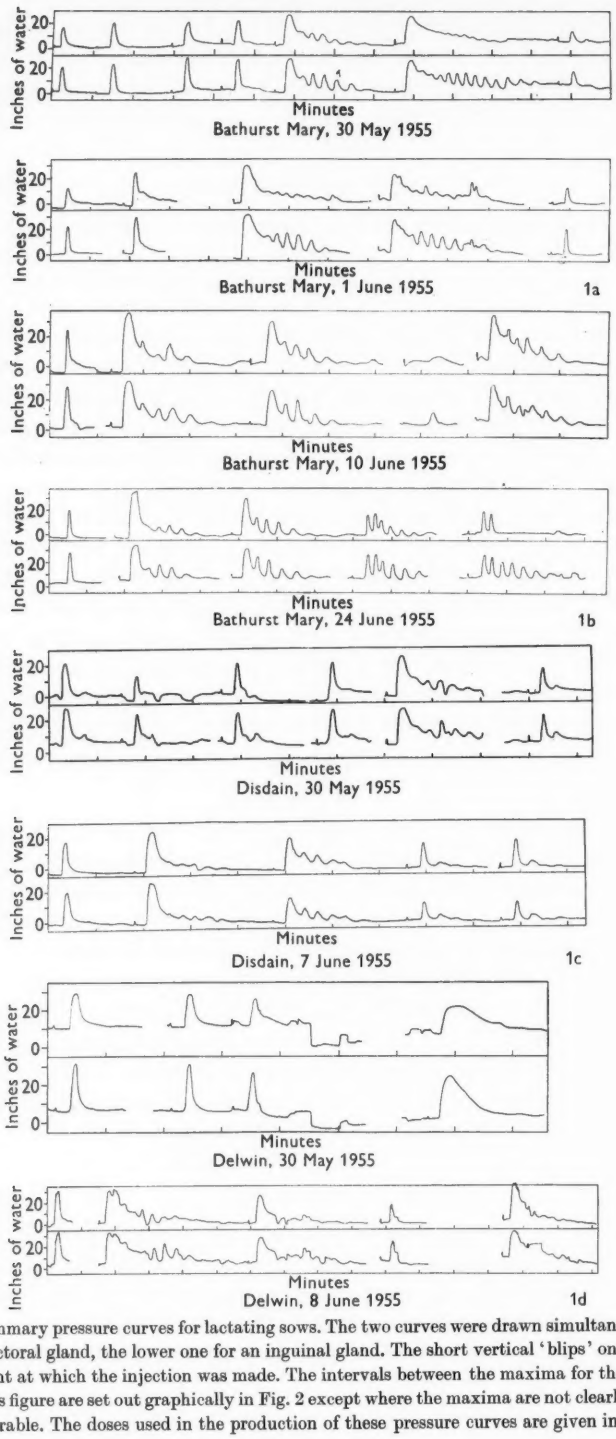

Fig. 1. Intramammary pressure curves for lactating sows. The two curves were drawn simultaneously, the upper one for a pectoral gland, the lower one for an inguinal gland. The short vertical 'blips' on the graph represent the point at which the injection was made. The intervals between the maxima for the pressure curves shown in this figure are set out graphically in Fig. 2 except where the maxima are not clearly enough defined to be measurable. The doses used in the production of these pressure curves are given in Table 1.

above the normal physiological level. The onset of oscillation varies considerably and in some cases starts almost immediately after the initial peak. Generally this initial peak is much higher than the rest of the curve. With the sow Bathurst Mary (24 June) a double injection was given twice, and as a result instead of a high initial peak followed by lower peaks of oscillation two roughly equal peaks were shown. It should be noted that in one of the recordings with this sow the cannula became blocked after the initial two pressure peaks and no further record was obtained. It is characteristic of all the oscillating pressure curves that the peaks of pressure fall steadily with time.

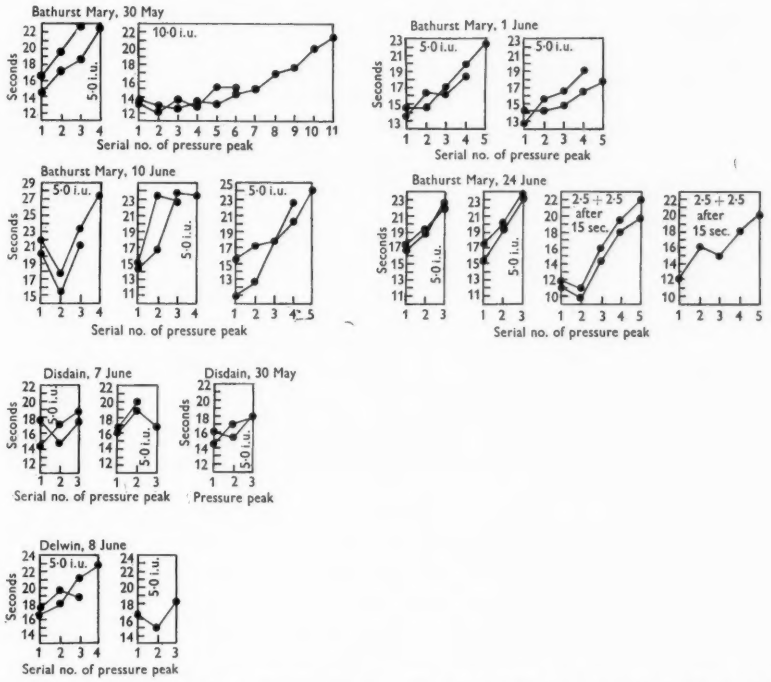

Fig. 2. The intervals between the pressure peaks shown in Fig. 1 are here plotted against the serial number of the pressure peak, thus indicating the change in frequency of the contraction with time.

In Fig. 2 the interval between peaks is plotted against the serial number of the peaks. This shows that there is a general tendency for the frequency of oscillation to fall. In some cases the interval decreased slightly at the beginning to increase steadily later on. The intervals ranged between 10 and 25 sec. in duration.

The forms of the pressure graphs plotted simultaneously are similar though the peaks of pressure do not synchronize. With Bathurst Mary the trends in rate are very close for the different glands.

The differences in circulation time between the glands recorded are shown in Table 1. According to Sisson & Grossman (4) the pectoral glands are supplied via the internal and external thoracic arteries, while the external pudic artery supplies the inguinal and abdominal glands. The interval between injection and the initiation of the pressure rise

is a fairly precise indication of the circulation time between the ear vein and the gland concerned. It is apparent from the figures in the table that the delay tended to be shorter with the foremost glands than with the rear glands though there were some inexplicable irregularities. However, the difference was small.

Table 1

			Delay (sec.)	
			Fore gland	Rear gland
Sow	Date	Dose (i.u.)	1st R	6th R
Bathurst Mary	30 May	0·25	10·1	10·4
		0·5	19·1	20·2
		0·5	17·6	18·4
		0·5	19·1	20·3
		5·0	12·7	14·6
		10·0	15·1	16·2
		0·5	18·3	18·7
	1 June	0·5	21·3	22·6
		0·5	13·9	14·7
		5·0	12·1	12·8
		5·0	18·0	18·8
		0·5	18·5	18·7
	10 June	0·5	18·2	10·2
		5·0	13·8	14·9
		5·0	19·7	21·7
		0·5	—	31·5
		5·0	15·2	16·7
	24 June	0·5	Not recorded	Not recorded
		0·5	28·6	29·0
		5·0	24·9	17·0
		5·0	15·3	16·6
		2·5 + 2·5 after 15 sec.	20·3	19·6
		2·5 + 2·5 after 15 sec.	20·6	22·4
		0·5	—	19·4
		Average	17·73	17·83
			1st R	4th R
Disdain	30 May	0·5	16·0	16·5
		0·5	16·5	15·7
		0·5	15·8	16·5
		0·5	14·7	14·4
		5·0	12·7	13·1
		0·5	15·3	15·2
	7 June	0·5	17·1	17·7
		5·0	15·6	16·1
		5·0	14·9	16·2
		0·5	17·9	18·0
		5·0	15·6	16·7
		Average	15·65	16·01
			1st L	Rear L
Delwin	27 May	0·5	15·2	16·4
		0·5	14·0	15·3
		0·5	16·2	18·1
		5·0	11·1	11·9
	8 June		1st R	3rd R
		0·5	12·3	13·3
		5·0	10·6	11·2
		5·0	12·9	14·2
		0·5	15·2	16·0
		5·0	11·8	12·4
		Average	13·26	14·31

Fig. 3 sets out a typical human intrauterine pressure curve following the injection of one unit of oxytocin intravenously. The pressure record was taken using a cannula inserted into the amniotic fluid. There are clearly certain similarities between intramammary curves and the intrauterine one. There is the same sudden rise in pressure followed by a delay in some cases before the onset of regular oscillation. Like the mammary

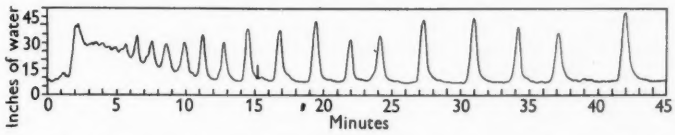

Fig. 3. A typical intrauterine pressure curve (human) showing the general nature of the periodic changes induced by an injection of oxytocin.

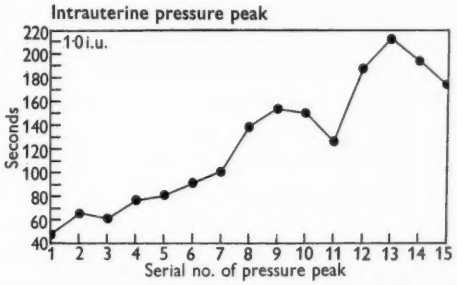

Fig. 4. The interval between pressure peaks for the intrauterine pressure curve in Fig. 3 is here plotted against the serial number of the pressure peaks in a way similar to that adopted in Fig. 3.

gland the uterus shows an increasing interval between pressure peaks with time (see Fig. 4). There are, however, some marked differences. The average pressure for the uterus is substantially higher than for the mammary gland and the pressure peaks do not fall with time. The intervals between pressure peaks are longer than with the mammary myoepithelium, and while in the latter oscillation ceases and the pressure returns to zero, at least in the sow, the uterus settles down to a series of sudden widely spaced pressure peaks.

Pharmacologically uterine muscle and mammary myoepithelium show marked similarities. Both are stimulated into action by both the peptides from the posterior pituitary, and other substances which stimulate the uterus muscle also have an effect on the mammary gland.

SUMMARY

Simultaneous intramammary pressure recordings were made on pectoral and inguinal mammary glands of the sow. Oxytocin was injected via the ear vein and the nature of the pressure curves resulting from quantities of hormone above physiological level studied. The frequency of the periodic contractions of the mammary myopethelium under these conditions was measured and compared with human uterine responses under similar conditions.

The writer is indebted to Professor H. McK. Carey of the National Women's Hospital, Auckland for the intrauterine pressure graph shown in Fig. 3. Acknowledgement is also made of the technical assistance given during the experiments by Mr E. D. O'Reilly.

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(4) SISSON, S. & GROSSMAN, J. D. (1940). *The Anatomy of the Domestic Animals*, pp. 756, 760. Saunders.

APPENDIX

The apparatus used for the pressure recordings made in this work is somewhat different from that used previously. Fig. 5 shows the pressure head which consists of a bronze bellows mechanism coupled to a Philips magnetic displacement pick-up. The bellows are

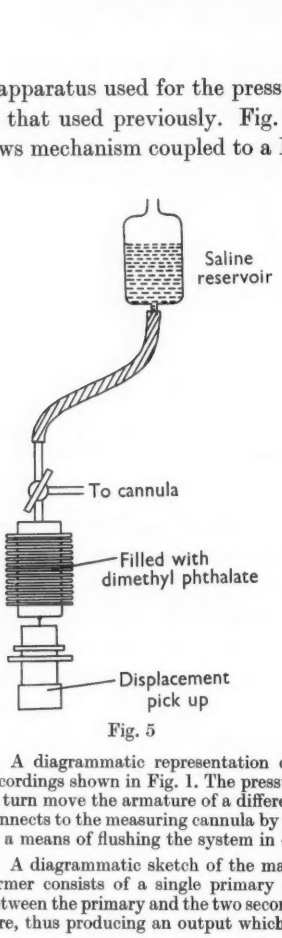

Fig. 5

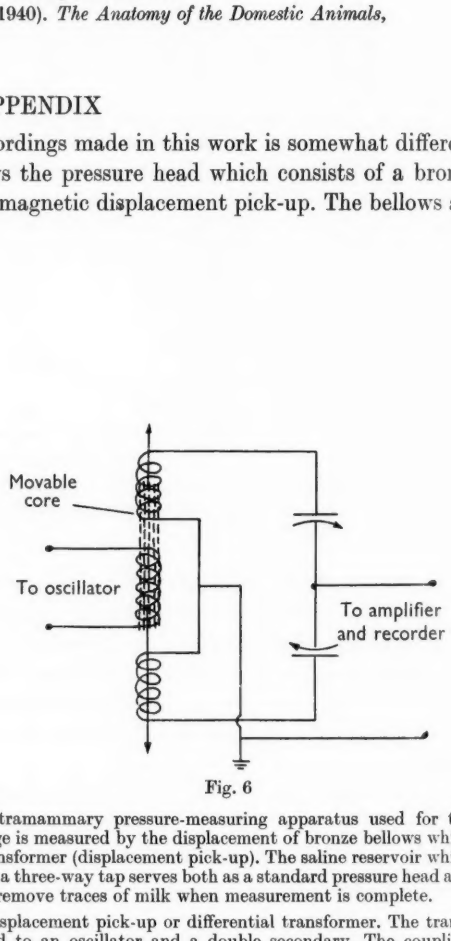

Fig. 6

Fig. 5. A diagrammatic representation of the intramammary pressure-measuring apparatus used for the recordings shown in Fig. 1. The pressure change is measured by the displacement of bronze bellows which in turn move the armature of a differential transformer (displacement pick-up). The saline reservoir which connects to the measuring cannula by means of a three-way tap serves both as a standard pressure head and as a means of flushing the system in order to remove traces of milk when measurement is complete.

Fig. 6. A diagrammatic sketch of the magnetic displacement pick-up or differential transformer. The transformer consists of a single primary connected to an oscillator and a double secondary. The coupling between the primary and the two secondary windings is altered by mechanical movement of the transformer core, thus producing an output which is proportional to the displacement.

filled with dimethyl phthalate, a non-corrosive liquid which is somewhat heavier than water. It should be noted that bellows of this type are unstable in the presence of saline, and corrosion may occur with a resultant loss in elasticity. The use of dimethyl phthalate eliminates this problem and it has been found possible to operate such bellows over a

period of many months without any sign of corrosion. A saline reservoir is connected to the bellows and to the rubber tube leading to the cannula via a three-way tap. The reservoir is maintained at a standard height above the cannula so that the recorder may be calibrated quickly by simply turning the tap to connect the reservoir direct with the bellows. Zero is set by turning the tap connecting the cannula to the bellows which is the normal recording position. Air bubbles may be removed and the whole system flushed with sterile saline by turning the tap to the position in which the reservoir, cannula, and bellows are connected together.

The Philips displacement pick-up is essentially a small transformer wired as shown in Fig. 6. The primary is connected to an audio-oscillator (a frequency of 6 kcyc. was used as the operating frequency). The secondary consists of two coils which are placed on either side of the central primary. The core is of 'Ferroxcube' and is moveable. As it moves out of one secondary into the other through the primary winding the coupling between the secondary windings and the primary is altered. If coupled in a bridge circuit as shown in Fig. 6, the whole system can be 'zeroed' by balancing the capacitative bridge. The output voltage caused by throwing the bridge out of balance by moving the core of the pick-up a small distance is closely related to the displacement. This means that the output voltage is a function of pressure in the arrangement shown in Fig. 5 and the device may be 'zeroed' electrically by altering the point of balance of the bridge with the variable capacitors. The bridge was made from two small portable radio-type tuning condensers of a capacity of approximately 300 $\mu\mu$F. coupled together mechanically so that as the capacity of one increases the other decreases. The unit was carefully screened.

The output from the bridge was taken to an amplifier similar to that described earlier (3). By the use of more negative feed-back the amplifier was made even less susceptible to changes in mains voltage, and was found to be entirely satisfactory for the purposes described. The output of the amplifier was fed through a germanium diode rectifier bridge to an Elliott strip-chart recorder with a 2 mA. movement. This type of recorder produces a rectilinear graph which is a distinct advantage over the curved charts used earlier. Two identical amplifiers and recorders were used with two pressure heads.

The reason for changing to the use of the bronze bellows pressure head instead of the original mercury manometer described earlier was primarily that the former has a very much better frequency response. The inertia of the mercury manometer is relatively high, while the bronze bellows equipment has a very much faster response. However, it would appear that for intramammary pressure measurements this refinement has had no other effect than to sharpen slightly the peaks of the pressure curves, a point which adds very little to the information obtained.

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637.127

681. THE CHEMICAL COMPOSITION AND THE CELL COUNT OF MILK

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(With 4 Figures)

For many years the view has been held by Malcolm and his colleagues (1,2,3) that a form of mastitis, sometimes referred to as subclinical or non-specific, to distinguish it from clinical mastitis, exists widely in milking cows. Recently Laing and he (4) have estimated that the proportion of milking cows in the west of Scotland suffering in this way is as high as 14%. The evidence for these views was based largely on the number of cells present in the milk. These workers recognized that in very early and very late lactation numerous epithelial cells may be present in a cow's milk because of the rapid changes taking place in the mammary tissue, and that these cells are not necessarily associated with disease. During the major part of the lactation period, however, the presence of large numbers of cells in the milk was taken to indicate some form of inflammation in the udder.

The early work was based on the measurement of the total number of cells present irrespective of the type of cell. Blackburn (5,6) extended this determination by differentiating between three types of cells, the lymphocyte, polymorph and epithelial cells. He considered (7) that a high total count in which the polymorph cells predominated was indicative of some pathological condition in the udder. It may be noted that polymorph cells are the main cellular constituent of pus. Blackburn's opinion was based on numerous histological examinations of udders from cows whose milk had been regularly examined for cell count during the immediately preceding lactation.

An important aspect of all this work was that a high cell count was by no means necessarily accompanied by the presence in the milk of the bacteria normally considered to be the cause of clinical mastitis. Indeed, not infrequently pathogenic bacteria were entirely absent.

Blackburn (unpublished data) found that those quarters of the udder that gave milk with a high cell count, particularly if high in polymorph cells, usually gave far less milk than a corresponding healthy quarter. There has, however, been little examination so far of the effect of this type of subclinical mastitis on the chemical quality of the milk, although the marked chemical changes occurring in the milk from cows suffering from clinical mastitis have, of course, been known for many years. In the course of an investigation which involved the chemical analysis of some 2000 samples of milk from individual cows in commercial herds, the opportunity was therefore taken to measure the total and differential cell counts of the samples, and to compare chemical composition and cell count. This comparison was later extended to samples of farm bulk milk.

EXPERIMENTAL

The milk from the individual cows came from 180 pairs of dams and their daughters located in 14 commercial herds of Ayrshire cows in south-west Scotland. Each cow was sampled six or seven times during its lactation at intervals of approximately 5 weeks, and samples from the complete evening and morning milks, mixed in proportion to yield, were taken for analysis. The samples were analysed during the day on which the morning sample had been taken and hence no change in chemical composition was likely. In view of the large number of samples involved, however, it was considered advisable to adopt the precaution of including a small quantity of powdered potassium dichromate-mercuric chloride preservative in each sample bottle. Bacteriological examination of the milk samples was therefore precluded, but this limitation was accepted since the object of this preliminary survey was simply to compare the chemical composition of milk from the complete 24 hr. milking with the cell count.

The samples of farm bulk milk were taken at a Scottish Milk Marketing Board creamery in Ayrshire at the rate of sixteen each week for 1 year. No attempt was made to sample the milk from the same farms each week although that occasionally happened. In an endeavour to avoid the milk from very small herds, since the quality of such milk might be considerably influenced by a few outstandingly good or bad cows, samples were taken only when the volume of the mixed evening and morning milk exceeded 50 gal. This general rule was relaxed for 3–4 weeks in September when the seasonal nature of the calving policy of the suppliers of the creamery caused a marked drop in yield. Some samples were therefore taken from bulks as small as 30 gal.

Analyses

The amounts of total solids, fat, solids-not-fat (by difference), crude protein, casein and lactose in the milk were determined by methods recently described (8).

RESULTS

The total cell count and the percentages of lymphocyte, polymorph and epithelial cells were observed under the microscope in a Breed smear by the technique described in the work previously mentioned (5). Although the amount of milk examined in this method is very small (0·01 ml.), the reproducibility of the result is satisfactory. This can be seen from the figures in Table 1 for five samples of milk with total cell counts ranging from 20,000 to 3,000,000/ml., each milk having been examined in triplicate.

Table 1. *The reproducibility of the results of the counting techniques*

Milk sample no.	Total cell count ('000/ml.). Replicate no.			Differential count (%). Replicate no. 1			2			3		
	1	2	3	P*	L*	E*	P	L	E	P	L	E
1	20	0	0		—			—			—	
2	100	80	130	50	0	50	60	0	40	30	0	70
3	270	300	420	55	0	45	63	7	30	69	0	31
4	950	1040	1080	71	3	26	75	3	22	68	1	31
5	2920	3040	3280	53	6	41	48	4	48	49	5	46

* P = polymorph cells. L = lymphocyte cells. E = epithelial cells.

Before examining the association between cell count, yield and chemical composition of the milk it is perhaps useful to establish the general quality of the milk supplies.

The milk from the individual cows, with which this survey is chiefly concerned, had an average chemical composition of 4·04% fat and 8·86% solids-not-fat (S.N.F.). Of the 2186 samples, 35 contained less than 3·0% fat and 320 had less than 8·5% S.N.F. (the presumptive legal minima). The average daily yield per cow of the samples from the individual cows was 34·6 lb.

The total cell count of these samples ranged widely, from less than 10,000 to 5,000,000/ml., but about 1500 samples, or more than two-thirds of the total number, contained less than 200,000 cells/ml. Only 140 samples had total counts of more than 1,000,000 cells/ml. Grouping the samples according to their total cell content, as in Table 2, showed significantly that when polymorph cells were present in the milk a much greater

Table 2. *The distribution of milk samples into groups of total cell counts, with those samples containing polymorph cells shown separately*

	Number of samples	
Total cell count	Containing polymorph cells	Not containing polymorph cells
0–50,000	0	684
60,000–100,000	0	440
110,000–200,000	170	208
210,000–300,000	166	25
310,000–400,000	105	8
410,000–500,000	73	4
510,000–600,000	37	4
610,000–700,000	37	8
710,000–800,000	31	3
810,000–900,000	18	2
910,000–1,000,000	21	2
1,000,000–2,000,000	91	8
2,000,000–5,000,000	35	1
> 5,000,000	5	0
Total	789	1397

proportion of such samples had high total cell counts. The average total cell count of the samples which contained polymorph cells was 650,000/ml., whereas of the larger number of samples which did not, the average total cell count was only 83,000/ml. As can be seen from Table 2, most of the samples in which polymorph cells were absent had total cell counts below 100,000/ml. For the samples in which polymorph cells occurred, i.e. above a total count of 100,000 cell/ml. there was a close linear relationship between the total count of the sample and its polymorph content. This was expressed by the equation

$$y = 0{\cdot}79x - 74{,}000,$$

where x = the total cell count and y = the polymorph cell count.

The farm bulk milk samples were fewer in number (823) and were analysed chemically only for fat and S.N.F. contents. The average composition was 3·83% fat and 8·76% S.N.F.; none of these samples contained less than 3·0% fat and 70 (9%) were below 8·5% S.N.F.

These samples contained rather more cells of all types than the milk from the individual cows, although only two samples had a total count of greater than 1,000,000 cells/ml. The

proportion of farm bulk samples with less than 200,000 total cells/ml. was 59% compared with 72% for the individual cow samples. Polymorph cells were present in rather more than half the total number of farm bulk samples, whereas they were present in only one-third of the individual cow samples.

It can be seen that the general quality of the individual cow samples was very good and that of the farm bulk samples good.

In the results which follow we consider that good preliminary evidence is found to associate high counts, of either total or polymorph cells, with lower than average S.N.F. contents, and in particular with lower than average lactose contents. Before accepting such evidence as possible cause and effect it was necessary to establish that a high cell count and, for example, a low lactose content were not merely the result of two physiologically normal phenomena. A rise in cell count, unassociated with disease, might possibly occur normally as lactation progressed and during most of that period the lactose content would normally be falling (8).

The effect of stage of lactation on cell count

The lactation period was therefore divided into fourteen groups of 20 days each and the average value of the cell counts, both of total and polymorph cells of the samples in each group calculated. The cows had not been sampled in what was expected to be their last month of lactation (although the last sample taken from a small number of non-persistent cows would come into this category) and hence the results are incomplete to that extent. Moreover, the number of samples in each group after about 220 days decreased rapidly and a few abnormal samples would therefore be more easily capable of affecting the average value for these groups.

A noticeable feature of the results was the considerable variation that existed between the milk of different cows in the relationships between cell count and composition. A measure of this has been provided by reporting the standard deviation of individual results about the mean of the group for each constituent. The standard deviations of the cell counts in the fourteen groups into which the lactation was divided ranged from 160,000 to 210,000 cells/ml., the smaller number of samples in the late lactation groups having the higher standard deviations.

The average total cell counts during the lactation period are shown in Fig. 1. Over the first 170 days the change in the cell count was small, the period between 70 and 130 days having the lowest values. From 170 to 270 days, the end of the sampling period, the total count increased slowly at first and then more rapidly. In Fig. 1 it can be seen that the values shown for the polymorph counts were obtained from only a third of the samples contributing to the total cell counts. Samples in this third had such high total cell counts (Table 2) that their polymorph fraction exceeded the total cell count of all the samples.

The average values of the polymorph count throughout the lactation period were somewhat more irregular than the total count, as might perhaps be expected if these cells represent the effect of disease. From a surprisingly high count in early lactation the number of polymorphs fell steadily to the 130th day of lactation. From this point onwards there was considerable variation in the polymorph count, with a tendency for it to rise in the later stages.

The increase in both total and polymorph count towards the end of lactation was caused mainly by a small number of samples which had counts greater than 2,000,000 ml.

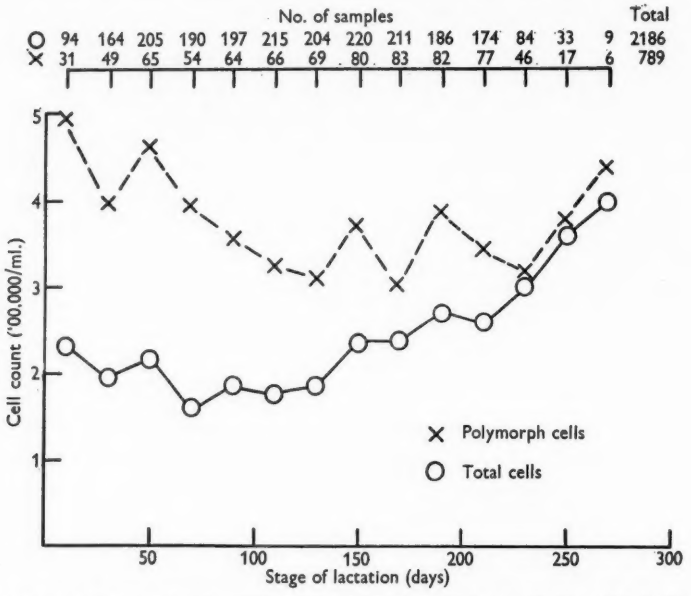

Fig. 1. The total and polymorph cell counts during the lactation period. (Only a third of all the samples contained polymorphs and the total counts for these samples were very much higher than the average total counts shown here for all the samples.)

The effect of age of the cow on cell count

It is well established (8,9) that as the age of a cow increases, the chemical quality of the milk declines. It is also known (8,9) that milk yield is negatively correlated with both the fat and S.N.F. contents, and since milk yield increases with the age of the cows, the quality of the milk of older cows would be expected to go down. It was of interest, therefore, to determine the relationship between age of cow and the cell count of the milk. This is shown in Fig. 2. The results do not relate to the same cows in successive lactations, but to samples taken in the same year from cows that were in lactations ranging from the first to the eleventh.

The total cell count of the milk rose steeply until the seventh lactation and then increased at a slower rate. It should be emphasized that the cows which were lactating for more than the third or fourth time were, in several aspects, a selected population. All the cows came from milk-recorded herds, and would therefore have been retained for their milk-yielding and butter-fat-producing capacity. That they had reached these advanced ages suggests that they had also a reasonable immunity to disease. However, in general farm practice all cows of similar age will, to a great extent, be selected at least for yield and general health.

As the ages of the cows increased, the polymorph cell count of the milk increased, and in samples from cows in their first, second, third and fourth lactations, the average

polymorph cell content, when polymorphs were present, was appreciably higher at any lactation number than the average total count of all the samples in that group. As can be seen in Table 3, the effect of the age of the cow on the proportion of milk samples containing polymorph cells was most marked, the proportion rising sharply after the second lactation.

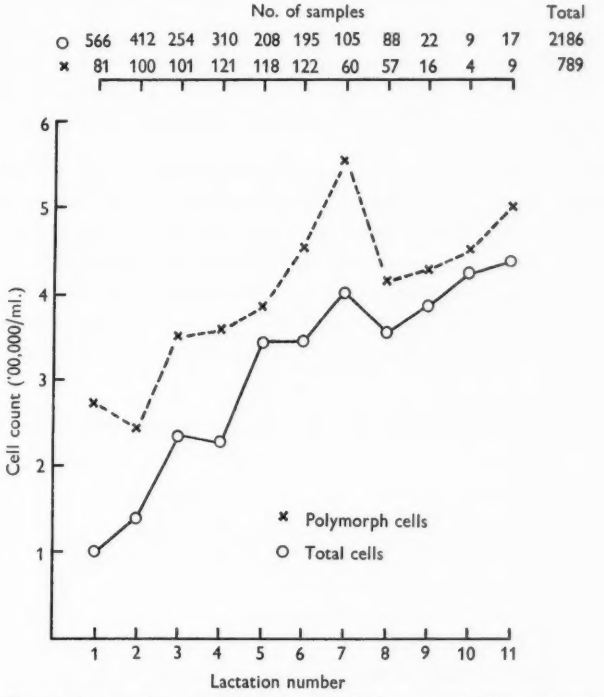
No. of samples
Total
○ 566 412 254 310 208 195 105 88 22 9 17 2186
× 81 100 101 121 118 122 60 57 16 4 9 789
Cell count ('00,000/ml.)
Polymorph cells
Total cells
Lactation number

Fig. 2. The total and polymorph cell counts of milk from cows in different lactations.

Table 3. *The effect of the age of the cow on the proportion of samples containing polymorph cells*

No. of lactations	1	2	3	4	5	6	7	8	9	10	11
% samples containing polymorph cells	14	24	40	42	57	63	57	65	73	33	53

Cell count and milk composition

With the large number of results necessary for a general survey of this type, their presentation is difficult. The method which has been adopted has been to divide the samples into groups differing in cell count by 50,000/ml. in the two lowest ranges and then by 100,000/ml., and to calculate the mean value of the yield and the fat, S.N.F., casein and lactose contents of these groups. The standard deviation of the individual values from the group mean has been calculated, and typical values are given in Table 4. It can be seen that there was a considerable spread at all levels of cell count above 50,000/ml. and that the spread increased, as expected, in the small groups of samples with high cell counts.

Table 4. *The standard deviations of individual samples from the group mean at different levels of total cell count (individual cow samples)*

Total cell count (cells/ml.)	No. of samples	Fat (%)	S.N.F. (%)	Casein (%)	Lactose (%)
0–50,000	811	0·54	0·08	0·08	0·08
60,000–100,000	412	0·55	0·17	0·28	0·20
110,000–200,000	344	0·48	0·35	0·27	0·36
410,000–500,000	70	0·54	0·36	0·21	0·31
710,000–800,000	32	0·63	0·43	0·33	0·30
910,000–1,000,000	19	0·38	0·46	0·27	0·32
>1,000,000	131	0·54	0·41	0·16	0·42

(*a*) *Total cell count*

In Fig. 3 the daily yield of milk per cow and the composition of the milk of the individual cow samples have been plotted against the total cell count. Above a cell count of 500,000/ml. the number of samples in each group decreased considerably, and this may account for the irregularities in some of the curves at the high counts.

It appeared that as the total cell count of the milk increased, the yield per cow fell appreciably when the average count exceeded 300,000/ml. In two high count groups the yield rose again, a result possibly caused by the small number of samples in both these groups.

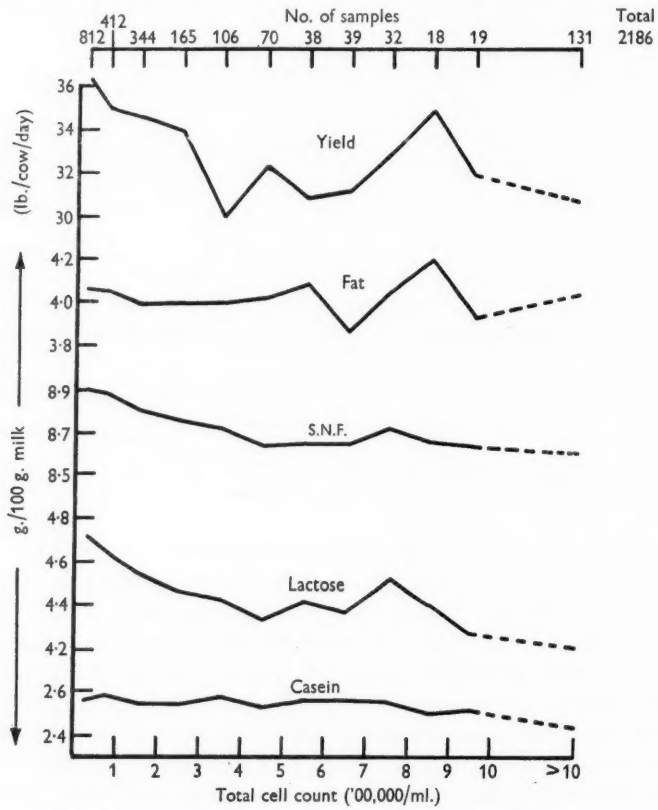

Fig. 3. The total cell count and chemical composition of the milk.

The fat content of the milk remained substantially the same at all levels of cell count, but there was a marked decline in S.N.F. content, from 8·9 to 8·6%, as the average cell count rose from 25,000 to 500,000/ml. This drop in S.N.F. was caused entirely by the decrease that occurred in the lactose content, the casein content remaining extremely constant up to an average count of 800,000/ml. Above this level the casein content also decreased. As with yield, an increase of cell count up to 500,000/ml. was associated with a much greater drop in S.N.F. and lactose contents than was the increase from 500,000 to 1,000,000 cells/ml. In the latter range there were, in fact, some increases in the average values of both constituents. A possible reason for this is discussed later but, again, these points are derived from much smaller numbers of samples than are those in the cell count range up to 500,000/ml.

When the milk samples were graded as 'normal' or 'high' according to whether their total cell count was below or above 100,000/ml., and then divided into those samples above or below the presumptive legal minimum of 8·5% S.N.F. (Table 5), it was clear that cell counts higher than this level militated against high S.N.F. contents.

Table 5. *The distribution of the cell counts in milk containing more and less than* 8·5% *solids-not-fat* (*individual cow samples*)

	Total cell count (% of samples in each range)	
S.N.F. range	Normal	High
Above 8·5%	65	35
Below 8·5%	37	63

Normal = milk containing less than 100,000 total cells/ml.
High = milk containing more than 100,000 total cells/ml.

Corresponding figures for the total cell count and the S.N.F. content of the farm bulk milk samples are given in Table 6. A very similar trend to that for the individual cow samples can be seen, a rise in count from 50,000 to 550,000 cells/ml. occurring at the same time as a fall in S.N.F. content of 0·13%.

Table 6. *The total cell count and the solids-not-fat content of farm bulk milk*

Group mean count (cells/ml.)	No. of samples	Mean S.N.F. content (%)
0–100,000	235	8·80
110,000–200,000	249	8·77
210,000–300,000	163	8·72
310,000–400,000	75	8·59
410,000–500,000	52	8·71
510,000–600,000	28	8·67
610,000–700,000	7	8·58
710,000–800,000	9	8·66
810,000–900,000	2	8·65
910,000–1,000,000	4	8·58
> 1,000,000	2	8·50

(*b*) *Polymorph cell count*

The polymorph cell count was calculated from the percentage of polymorph cells in the total count; the samples in any level of polymorph count were therefore derived from total counts of higher levels.

The daily yield per cow and the chemical composition of the individual cow samples have been plotted against the polymorph cell count in Fig. 4 to determine whether a differential cell count gave any clearer indication of milk quality. The results suffered from the same wide spread that was shown in Table 4 for the total counts, partly because

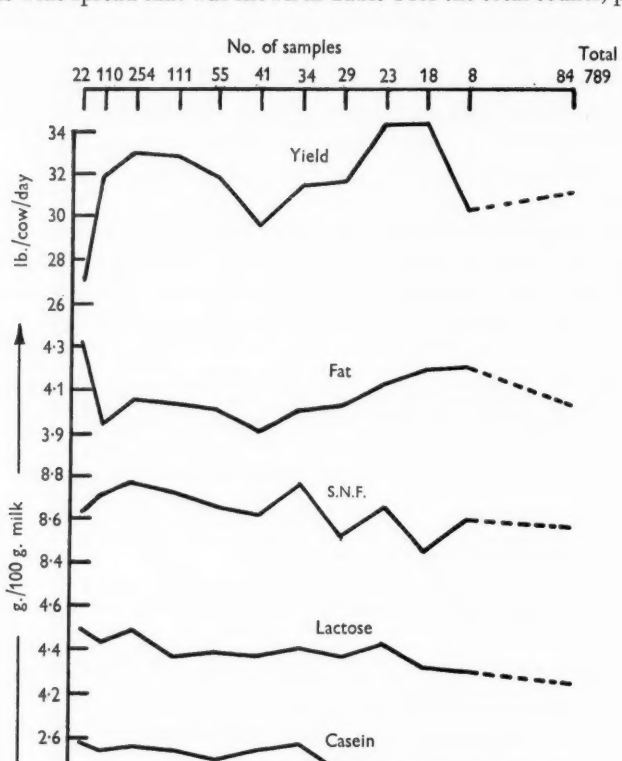

Fig. 4. The polymorph cell count and chemical composition of the milk.

of the smaller number of samples in each group, but also because of the varying response to subclinical mastitis of individual cows as indicated by the cell count of their milk. The curves are therefore somewhat more irregular than the corresponding curves in Fig. 3 for composition and total cell count. In general, an increasing polymorph cell count appeared to be less associated than total count with any progressive decline either in yield or chemical composition. The lactose content decreased appreciably (0·13%) between 25,000 and 250,000 polymorph cells/ml. and the S.N.F. content fell by about the same amount between 150,000 and 450,000 polymorph cells/ml. It is interesting to note that above 600,000 cells/ml. the casein content also fell and that the casein number (the percentage of the total nitrogen of the milk that was present as casein) fell from 78 to 76; these results were, of course, from a very small number of samples. The general levels of yield, S.N.F. and lactose contents were lower than at similar levels of total count.

The 823 farm bulk samples contained 433 samples with a polymorph count which was mainly concentrated in the three subgroups from 0 to 300,000/ml. There was a slight decrease in S.N.F. content from 8·73 to 8·63% as the count increased from 50,000 to 1,000,000/ml.

DISCUSSION

In the results presented here no attempt has been made to postulate what the cell count of milk from completely healthy cows might be. This will be considered fully in a later paper in which the yield, chemical composition, bacteriological examination and cell counts of the milk from four quarters of individual cows will be reported. It can, however, be said that a total count of less than 10,000 cells/ml., with a complete absence of polymorphs, appears to be characteristic of milk from a healthy udder. Of the present samples from individual cows, only 10% were in that category, although 37% had less than 25,000 cells/ml. and 56% had less than 100,000 cells/ml. Up to a total count of 100,000 cells/ml. polymorph cells contributed only a negligible fraction, almost all the cells being epithelial in origin. Above this figure, the proportion of polymorph cells increased rapidly with increasing total count. From general experience, cows with milk from a full milking having less than 100,000 cells/ml. would not, therefore, be considered to be suffering from subclinical mastitis.

In milk from cows suffering from clinical mastitis the S.N.F. content is usually low. The lactose content of such milk is particularly affected, whilst the casein content and the casein number are also low (10, 11). In the present samples the S.N.F. and the lactose contents both decreased, by 0·25 and 0·38%, respectively, as the total cell count increased to 500,000/ml., but the casein content was reduced only in those samples which had a polymorph cell above this level. The rise in both total and polymorph cell count in the last quarter of the lactation would coincide with the normal fall in lactose content, but equally at this time both the S.N.F. and casein contents normally rise appreciably. The fall in the S.N.F. and casein contents at the higher levels of cell count therefore appear to be connected more with the cause of the cells in the milk than with normal lactational changes. Thus the chemical results are consistent with either the early stages, or a mild form, of what in a later or more virulent stage would be diagnosed as clinical mastitis. It should be emphasized that none of these milk samples contained any of the clots associated with the clinical form of the disease.

The fat content, on the other hand, did not appear to be closely connected with the cell content of the milk, although in the small group of samples containing on average 25,000 polymorph cells/ml. there was a surprisingly large drop in yield and a complementary rise in fat percentage.

It also seems probable that the cows giving milk with total cell counts above about 250,000/ml. were yielding less than they potentially could and that the deficiency may have amounted to 3–5 lb./cow/day.

Because the proportion of polymorph cells in the total count was large when the total count was high, little extra indication of the chemical composition was given by the differential measurement of the cell contents. The polymorph count would be useful, when the stage of lactation of the cow was unknown, to differentiate early and late lactation milks in which the epithelial cell content might be high.

It is interesting to speculate why the fall in S.N.F. and lactose contents was less pronounced above a level of 500,000 total cells/ml. in the milk than below it. Some milk samples contained large numbers of total cells and even of polymorph cells and yet maintained a relatively high chemical composition. A possible explanation is afforded by a recent observation (Blackburn, unpublished) that the lesions in the udder tissue of cows which have given milk with high cell counts are of two distinct types. In one the lesions are mild but widespread throughout the udder tissue, so that the composition of the milk might well be considerably affected. In the second type the lesions are severe but occur in relatively few foci. This second type of damage may therefore lead to milk of high cell count but, because of the small area of udder involved, of fairly normal chemical composition. This hypothesis is now being explored.

The farm bulk samples had cell contents which were higher than had been expected, with fewer of them occurring in the low count range of 10,000–100,000/ml. than those of the individual cow samples, and the results confirm Laing & Malcolm's(4) view of the widespread incidence of the disease. These higher cell counts, of both total and polymorph cells, appear to be reflected in the lower S.N.F. contents of the bulk milks compared with the milk from individual cows, which were drawn from a wider area. The very similar distribution of cell counts in the farm bulk samples, all accepted at the receiving creamery, is additional evidence that such cell counts are probably typical of milk from commercial herds and that such milk is normal in appearance. The farmer has therefore no obvious way of knowing that the quality of the milk from some of his cows is inferior to what, in the absence of the subclinical mastitis, it could have been.

It seems reasonable to conclude, therefore, that the type of udder inflammation which, once lactation is established, results in the presence of more than 25,000 cells/ml. in the milk leads to a marked fall in the lactose content of the milk and that this results in lower S.N.F. contents.

SUMMARY

1. The mixed evening and morning milk from 360 cows, all sampled six times during their lactation, was analysed chemically and for its cell content. A total and a differential cell count were made. Similar analyses were made on 823 samples of farm milk taken at a receiving creamery.

2. The total cell counts varied from less than 10,000 to 5,000,000/ml. and there was considerable variation in the chemical composition of samples containing similar numbers and types of cells.

3. The proportion of polymorph cells in the total count increased rapidly with increasing total count.

4. Stage of lactation had little effect on the number of cells present in the milk, but samples from cows in very early and very late lactation had been avoided. The total cell count was lowest from the 70th to 130th day of lactation with a tendency to rise after the 130th day.

5. Both the total and the polymorph cell counts of the milk increased sharply with the lactation number of the cow until the seventh lactation and then remained at a fairly constant level.

6. As the total cell count increased to an average value of 500,000/ml. there was a fall in the daily yield of milk per cow and a marked fall (0·25%) in solids-not-fat content.

The latter was the result of a steady drop in the lactose content, the casein content being affected only by counts approaching 1,000,000/ml. An increasing cell count was without effect on the fat content. Possible reasons for the relatively small effect on chemical composition of high cell counts in some milks is discussed.

7. It is concluded that the presence of subclinical mastitis, as evidenced by the cell count of the milk, leads to a lowering of the solids-not-fat content.

The authors wish to thank their assistants in the Technical Chemistry and Veterinary Pathology Departments, who did most of the practical work.

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682. SOME RESULTS ON THE VARIABILITY OF THE CLOT-ON-BOILING TEST*

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Experiments on the reproducibility of results obtained with the clot-on-boiling (C.O.B.) test were reported by Rowlands, Barkworth, Hosking & Kempthorne (1). These workers found that, with 3 hr. observations, the end-points of quintuplicate subsamples occurred at the same testing time with 70% and within two successive testings for 93% of the samples examined. Rowlands *et al.* (1) also demonstrated that many samples with no sign of precipitation at one test could show heavy clotting at the next test carried out 3 hr. later, and this indicated that testing at more frequent intervals than 3-hourly was required for accurately estimating the time to first clotting with the C.O.B. test.

Investigating the minimum time interval within which the change from absence of clotting to presence could be detected with the C.O.B. test, Hosking & Margaroni (2) found that, testing at 15 min. intervals, the change occurred with successive tests for forty-seven out of fifty samples examined; the results (2) were reported by Rowlands *et al.* (1). These results demonstrated that, for variability studies on the C.O.B. test, the testing interval should not greatly exceed 15 min. when the storage temperature of the milk samples is in the region of 20° C.

The object of the two brief experiments with the C.O.B. test, reported below, was to obtain some preliminary information on the worker-to-worker variability of estimates and the reproducibility of results on one sample by one worker when tests were carried out at 15 min. intervals.

EXPERIMENTAL METHODS

In Exp. 1, series of C.O.B. tests were done on nineteen samples and each tube was examined by three people. In the following experiment (Exp. 2), one person carried out C.O.B. tests on twenty-four sets of five subsamples, each set having been drawn from one original sample.

For both experiments samples of morning milk were obtained from local creameries, brought to the laboratory by 1 p.m. on the day of production and stored at atmospheric shade temperature until 5 p.m.

Experiment 1. The samples consisted of approximately 60 ml. raw milk in 3 oz. rubber-stoppered glass bottles. At 5 p.m. on the day of production the samples were removed from the shade cabinet, vigorously shaken and then stored in a water-bath at 20° C. for the remainder of the experimental period. Each sample was again well shaken at 9 a.m. on the next day, and afterwards gently agitated at each testing time before the milk required for testing was withdrawn with a pipette. At hourly intervals from 9 a.m. the

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alcohol precipitation test was carried out until a precipitate was observed, and then a C.O.B. test was carried out at 15 min. intervals until clotting.

At each C.O.B. test interval, any one of workers A, B or C carried out the test and then all the three workers, in haphazard order, inspected the one tube of heated milk and privately recorded their own results. No discussion of C.O.B. results took place before the series of tests on a sample were completed.

Experiment 2. Samples of 300–350 ml. milk were obtained in 1 pint screw-topped blood-transfusion bottles. At 5 p.m. on the day of production, after shade-temperature storage, each sample was shaken and five subsamples of about 60 ml. were poured consecutively, in order corresponding to bottle number, into 3 oz. rubber-stoppered bottles previously labelled with the numerals 1 to 5. All subsamples were then placed in the 20° C. water-bath and all subsequent treatment was the same as in Exp. 1. As soon as a precipitate with the alcohol test was observed with any one subsample, the C.O.B. test was applied to all five subsamples taken from the same sample. Milk for each C.O.B. test was withdrawn, in order coinciding with bottle number, from each subsample in turn, and the inspection of the replicate tests was carried out in the same sequence.

Worker A carried out all laboratory operations, including the examination of all tubes of heated milk and recording of test results.

Alcohol precipitate test. To 1 ml. milk in a $6 \times \frac{5}{8}$ in. test-tube was added 1 ml. alcohol solution, 68% (v/v) ethyl alcohol, specific gravity 0·895 at 60° F., with bromo-cresol purple as indicator. The milk and alcohol solution mixture was shaken and examined for a precipitate. This test was used solely to give warning of the onset of changes in the milk expected to affect the C.O.B. test.

Clot-on-boiling (C.O.B.) *test.* A quantity of milk, approximately 2 ml. in Exp. 1 and 1 ml. in Exp. 2, was pipetted into a $6 \times \frac{5}{8}$ in. test-tube which was then placed in boiling water for 5 min. On removal from the water the tube was, without shaking, tilted to obtain a thin film of milk which was examined for a precipitate. In Exp. 1 the degree of curdling was assessed and recorded as follows: no precipitate, −; doubtful precipitate, .; slight but definite precipitate not confined to the cream layer, +; heavy clots but milk still liquid, ++; milk solid, +++. In Exp. 2 the precipitate was graded: absent, −; doubtful, .; present, ×.

Workers. A, B and C took part in Exp. 1 and worker A only in Exp. 2. Worker A had used the C.O.B. test more extensively than either B or C, both of the latter having had only limited experience of this particular test. For practice before the first experiment started, the C.O.B. test was carried out at intervals on about ten samples, the tubes were examined and results immediately discussed by all workers.

RESULTS

The time from 5 p.m. on the day of sampling to precipitation was used for analysis. Times to stage +, ++ and +++ were analysed for samples examined in Exp. 1: when a recorded − was followed by ++, the + was assumed to have occurred midway between the two readings, and similarly for ++ when successive readings were + and +++. In Exp. 2 only the time to × was considered as there was no grading of clotting intensity, and tests were generally discontinued after the second successive recording of a precipitate.

Experiment 1. Complete results were obtained for nineteen samples examined by workers A, B and C in March and April 1950.

The mean values for times to precipitation +, ++ and +++ are given in Table 1, and it can be seen from these results that close agreement between the recordings of the different workers occurred at all stages of clotting.

Table 1. *Mean values and their standard errors for time in hr. to different intensities of precipitation with the clot-on-boiling test, results recorded by three workers*

	Worker				
Precipitate	A	B	C	S.E.*	Mean
Slight (+)	19·38	19·39	19·41	0·016	19·39
Heavy (++)	19·77	19·81	19·79	0·035	19·79
Solid (+++)	20·76	20·83	20·80	0·037	20·80

* Standard error with 36 degrees of freedom.

The mean squares in the analysis of variance for the times in hours to the three clotting intensities are shown in Table 2. The analyses indicate that the differences between workers were not significant ($P > 0{\cdot}05$) at any level of precipitation. Standard deviations of single observations, estimated from the samples × workers interaction term given in Table 2 in hours, increased from 4·2 min. for times to stage + to 9·1 and 9·6 for ++ and +++ respectively.

Table 2. *Analysis of variance of times to precipitation in hr.*

		Mean squares		
	Degrees of freedom	Clot, +	Clot, ++	Clot, +++
Samples	18	11·5772	11·7555	13·4845
Workers	2	0·0052	0·0074	0·0208
Samples × workers	36	0·0049	0·0230	0·0255

Table 1 also shows that, on average, increased intensity of clotting occurs fairly rapidly after the onset of curdling, but the time lapse between successive recorded stages of precipitate differs with stages, from + to ++ averaged 0·40 hr. and from ++ to +++ 1·01 hr. The time interval between different recorded levels of precipitate, however, tended to vary widely from sample to sample and the minimum, maximum and approximate mean times required for increased clotting, according to the curdling scale used in this experiment, are given in Table 3.

Table 3. *Time interval between the recording of different stages of precipitation with the clot-on-boiling test; samples stored at* 20° C. *and tested at* 15 *min. intervals*

		Differences between clotting times (min.)		
		Minimum	Maximum	Mean (approx.)
Last recorded −	to first recorded +	15	15	15
Last recorded −	to first recorded ++	15	120	40
Last recorded −	to first recorded +++	30	240	100
First recorded +	to first recorded ++	15	105	25
First recorded +	to first recorded +++	15	225	85
First recorded ++	to first recorded +++	15	120	60

Table 3 shows that the change from no precipitate to slight, but definite, precipitate occurred within 15 min., one testing interval, for all the samples examined; but no such constant period elapsed between the change from + to ++ or +++. Under these experimental conditions, the change from − to +++ took from 30 to 240 min., but the sample here taking the longest time appeared exceptional as each of the remaining eighteen took 135 min. or less.

Inspection of the original records for the nineteen samples showed that no doubtful recording was made, and for only two samples was the first clot not recorded at the same time by all workers. Three samples examined by worker A, four by B and four by C had no + entry and of these, with successive clots − and ++, the three noted by A were similarly recorded by B and C.

Experiment 2. Results were obtained for twenty-four samples, tested and examined by worker A in May and June 1950.

The frequency distribution of the range of times between five replicate subsamples, drawn from the same milk sample, to reach a definite precipitate, ×, with the C.O.B. test are given in Table 4.

Table 4. *Frequency distribution of the range of times to first precipitate* (×) *with the clot-on-boiling test for quintuplicate subsamples*

Range of mean times to × (hr.)	Difference between earliest and latest × (min.)					Total samples
	0 or 15	30 or 45	60 or 75	90 or 105	120	
17·7–25·7	14	5	3	1	1	24

Table 4 shows that the agreement between results of replicate subsamples is generally satisfactory. The time to × for five replicates occurred within 15 min. with 58% of the samples (Table 4), within 30 min. with 67%, within 45 min. with 79% (Table 4), and within 60 min. with 88%; the range in times to end-point exceeded 1½ hr. for one sample only out of the twenty-four examined.

With respect to individual sets of replicate results, subsample 5 had a longer time to precipitate than subsample 1 with thirteen out of the twenty-four samples, equal with seven and shorter with four; these results indicated that order effects of a variable nature may be present. The possibility of order effects was, however, ignored when calculating the standard deviations of replicates on which Table 5 is based.

Table 5. *Frequency distribution of the standard deviation of times to first precipitate* (×) *with the clot-on-boiling test for quintuplicate subsamples*

Range of mean times to × (hr.)	Standard deviation (min.)				Total samples
	0–15·0	15·1–30·0	30·1–45·0	45·1–60·0	
17·7–21·7	12	2	0	0	14
21·8–25·7	4	3	2	1	10
17·7–25·7	16	5	2	1	24

Table 5 shows that two-thirds of the samples had a between-replicate standard deviation of time to precipitation of not more than 15 min., i.e. of one testing interval. The distribution shown in Table 5 also indicates that the standard deviation may increase with increasing mean time to precipitation. The regression of the between-replicate

standard deviation (y hr.) on the mean time to precipitation (x hr.) was significant ($P=0{\cdot}05$) and the calculated regression equation was $y=0{\cdot}24+0{\cdot}04\,(x-20{\cdot}72)$, the slope of the line having a standard error of 0·018 with 22 degrees of freedom.

Among the 120 replicates, obtained from twenty-four samples used in this experiment, ten replicates had one recorded doubtful between the last − and first ×, four had two and one had three doubtfuls; there were also nine instances when a single doubtful was recorded between the first and second ×.

DISCUSSION

Conclusions based on the results obtained in these two experiments are of limited applicability because small numbers of samples were examined at one laboratory only and the range of times to C.O.B. was restricted to between 16 and 26 hr. for approximate conformation with normal laboratory working time.

Earlier workers, for example Hosking & Margaroni (2) and Thomas & Jones (3), have suggested that there can be some initial difficulties in recognizing the onset of clotting with the C.O.B. test which may be generally overcome by experience of the test. Similarly, in Exp. 1 the good agreement between workers at the recording of first precipitate and the absence of doubtful readings demonstrated that early stages of precipitate may be determined with reasonable certainty. Although the number of doubtful recordings made in Exp. 2 seemed to oppose this concept, it was thought that many of these doubtfuls resulted from the worker's over-caution.

In Exp. 1 worker differences at the recording of first + which involved discriminating between absence and presence of a fine precipitate may have been expected to occur rarely, whereas differences at + + and + + + could have easily occurred as these were essentially more difficult decisions in that the extent of clotting was being assessed; the results (Tables 1 and 2) show that better agreement between workers was obtained at the stage + than + + and + + +, although, at all stages, differences between workers were small.

Since, at each level of precipitation, only small differences were observed between clotting times as recorded by individual workers, the times required to progress from − to +, to + + and to + + + may be largely regarded as characteristic of the sample examined. The results from Exps. 1 (Table 3) and 2 indicated that the onset of curdling with the C.O.B. test generally occurred very rapidly, the change from no precipitate to slight curdling often taking place within 15 min.: results in agreement with those published by Rowlands *et al.* (1). Exp. 1 results (Table 3) have also shown that the time lapse between − and + + or + + + varied considerably with different samples, and such sample-to-sample differences would detract from the efficacy of one simple interpolation scheme, such as used by Rowlands *et al.* (1), for estimating particular clotting times from observations at infrequent intervals. It seemed, therefore, that a good estimate of the actual time to clotting of a given intensity, after storage in the 20° C. region, may have been obtained with the C.O.B. test carried out at 15 min. intervals, but any increase in the testing intervals would have led to corresponding decrease in the reliability of the estimated clotting time.

The between-replicate variation studies of Exp. 2 suggested that the test was highly reproducible with this frequency of testing for most samples, see Table 5, particularly for

those with times to end-point of up to 22 hr. when stored at 20° C. The regression relationship found in this experiment supports the findings of Rowlands *et al.* (1), testing 3-hourly and storing samples at 22° C., who observed that the larger between-replicate differences occurred with milks having the longer mean times to curdling.

With C.O.B. testing at 15 min. intervals, single doubtful recordings between absence and presence of precipitate were reported by Rowlands *et al.* (1) for 6% of fifty samples and, in Exp. 2, one or more doubtfuls were recorded for 12·5% of the 120 sample-replicates examined, but for the reason already given this percentage, 12·5, may be excessive. In Exp. 1 there were no doubtful recordings, but a slight precipitate was not invariably recorded at the same testing time by all workers suggesting that a short period existed in which doubtfuls could have been encountered. In all three experiments mentioned here a doubtful class, of equal standing with the absence and presence categories, was included in the prescribed clotting scale. That doubtfuls were recorded so infrequently suggests that the transition period, during which the state of the heated milk samples may be visibly different from that required for definite non-curdling and curdling categories, is short and generally less than 15 min.

The change in milk cannot be expected to occur instantaneously and testing time must inevitably coincide with the transition period on a number of occasions. Doubtful precipitates may then be experienced because of normal observer limitations in seeing the very fine precipitates which probably occur between the recognized − and +. Since the transition period of discernible change appears to be brief, it is suggested that were the clotting scale restricted to absence or presence of precipitate for C.O.B. tests carried out at pre-determined times, only a very small proportion of tubes examined could not, with reasonable certainty, be graded as negative or positive.

SUMMARY

The C.O.B. test has been carried out at 15 min. intervals on raw milk samples stored at 20° C. to examine worker and replicate variability. Within experimental limitations, the differences between workers were not significant ($P > 0{\cdot}05$) and, in general, good agreement was found between the five replicates examined for each milk sample, coefficients of variation less than 4·0%. The change from absence to presence of a precipitate generally occurred with successive tests, but there were indications that the times to increased intensity of clotting differed with samples.

Captain H. Barkworth was responsible for the plan and design of these experiments which were carried out by members of his staff at Shardlow.

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683. FACTORS INFLUENCING THE VITAMIN CONTENT OF MILK FAT

VI. THE EFFECT OF REDUCED CAROTENE INTAKE

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(With 2 Figures)

The relationship between the daily carotene intake of cows and the levels of carotene and vitamin A in their milk fat has been investigated by a number of workers (e.g. (1,2,3)). Clear relationships have been established at lower levels of carotene intake, but it is apparent that there are maximum levels in the milk fat which cannot be exceeded by feeding carotene as the sole source of vitamin A. Most workers find these levels equivalent to about 50–60 i.u./g. fat. There is, however, less general agreement on the actual minimum carotene intake necessary to attain these maximum milk fat levels, and figures ranging from 0·3 g (1) to 2·5 g. (3) of carotene per day have been suggested.

Vitamin A potencies varying between 50 and 60 i.u./g. fat have been reported for New Zealand milk fats produced during the late autumn, winter and early spring, but the potency of summer milk fat has been found to be much lower (30–35 i.u./g. fat) (4). On the basis of overseas work these lower potencies might be expected to be associated with a lower carotene intake, but this is not the case since a recent survey (5) has shown that the carotene content of typical dairy pastures in New Zealand is uniformly high throughout the year and that although occasional lower values were encountered the daily carotene intake of cows on these pastures is normally in the order of 4–8 g./day. This is considerably in excess of even the highest levels referred to above as being adequate for the production of milk fat of maximum vitamin A potency, and is in the range where milk fat carotene and vitamin A would be expected to be independent of carotene intake.

It appears, therefore, that the utilization of carotene from New Zealand summer pastures is in some way impaired, resulting possibly in a different relationship between carotene intake and milk fat potency from that reported overseas. Accordingly, experiments were carried out to determine more precisely the nature of this relationship and also to investigate the utilization of an alternative source of provitamin (carotene in arachis oil).

EXPERIMENTAL

Experiment 1. Four pairs of monozygous twins, predominantly Jersey, from the herd of The Dairy Research Institute (N.Z.) were used in the investigation which was commenced in January 1956, when the levels of carotene and vitamin A in the milk fat were at a minimum. The animals were grazed out-of-doors on typical dairy pastures which at the time of the year were predominantly white clover with a small proportion of ryegrass and cocksfoot. During the 7-day pre-treatment period (period 1) all animals were on pasture

and were permitted to graze to appetite each day on an area which was limited by means of the electric fence. For the duration of the experiment four cows were offered this area of pasture each day. Their twin mates were then restricted for 8 days to one-half of this area per day (period 2), for 8 days to one-quarter of this area per day (period 3), and finally, for 10 days they were deprived of pasture almost completely (period 4). The eight animals then ran together on pasture for a further 15 days (period 5). During the periods of restricted pasture intake, the balance of the calculated requirements for maintenance and lactation (6) was provided in the form of hay and of a meal consisting of equal parts of linseed meal and crushed barley offered in the field simultaneously with the pasture which was rationed throughout the day to ensure a relatively uniform intake of pasture and meal.

Experiment 2. To investigate the utilization of an alternative source of carotene, two pairs of identical twins were used. Two animals remained on pasture throughout the experiment, while their twin mates were fed out-of-doors on concentrates and hay for 18 days (period 7). During the last 9 days of this period (period 7*b*) the meal-fed animals were both drenched twice daily, after the morning and evening milking, with a solution of carotene (synthetic β-carotene as supplied by L. Light and Co. Ltd.) in arachis oil to give a daily intake of 750 mg. carotene in 150 ml. oil. The four cows were then maintained on pasture for a further 12 days (period 8).

All cows were milked normally twice a day, milk samples being collected at the evening milking. Milk fats were prepared for assay essentially as described in part III of this series (7) and vitamin A and carotenoids were estimated using the method of Thompson, Ganguly & Kon (8).

Pasture and feed samples were taken at intervals of about 7 days and were assayed for carotene using the method described by Worker (9).

RESULTS

Experiment 1. Throughout the experimental periods the level of carotene in the pasture remained relatively constant at 500–550 μg./g. dry matter. Assuming a feed intake of 2½% body weight, this gave a daily carotene intake of about 5 g. when the animals were on full pasture (periods 1 and 5). Allowing for the carotene content of the dry feed, the daily intake was approximately 2·5 g. during period 2, 1·3 g. during period 3, and 0·1 g. during period 4 when pasture contributed only about 1% of total feed intake.

Average milk fat vitamin A and carotenoid levels for the four pairs of animals are shown in Fig. 1. All animals showed similar trends and similar sample fluctuations which in many cases exceeded treatment differences except during period 4. However, it seems apparent that a reduction in carotene intake to one-half normal had no effect, and to one-quarter normal only slight effect, on the levels of vitamin A ester or alcohol. When the carotene intake was further reduced to 0·1 g, vitamin A ester was depressed on the average by about 1·5 μg./g., but vitamin A alcohol was not appreciably affected. During period 2 the average level of carotene in the milk fat was reduced by about 1 μg./g. fat and during periods 3 and 4 by about 2 μg./g. fat. Treatment had little effect on xanthophyll levels until period 4, when a depression of about 1 μg./g. fat occurred.

Milk fat vitamin A ester and carotenoid levels returned slowly to normal during the post-experimental period, vitamin A ester being somewhat higher for several days in the milk fat of the animals whose carotene intake had previously been restricted.

Experiment 2. The results of the experiment in which two animals were dosed with carotene in oil as an alternative source of carotene are presented in Fig. 2. During period 7 the treated animals received the same 'low-carotene' feed as was used during Period 4 (Exp. 1) and their daily carotene intake was again approximately 0·1 g. As in the previous

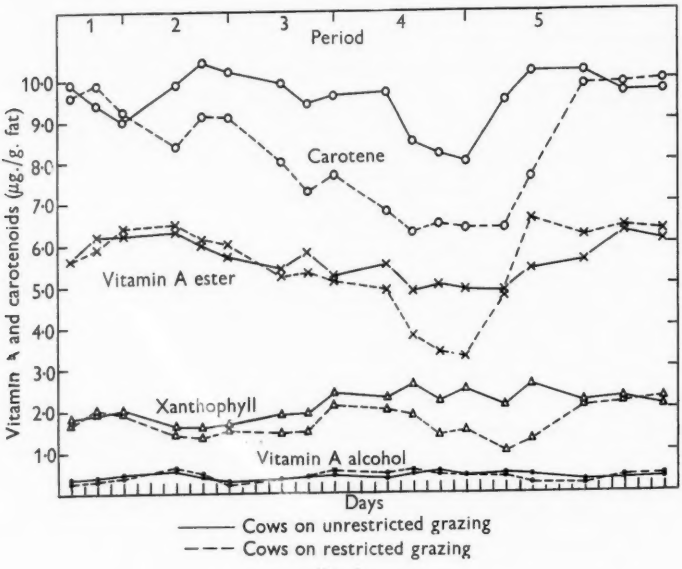

Fig. 1

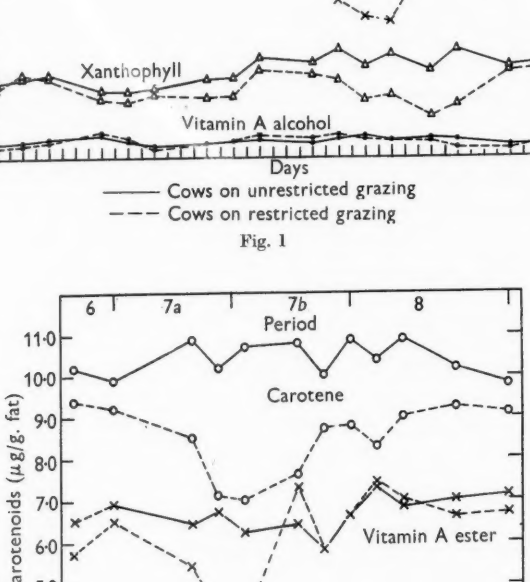

Fig. 2

experiment, this level of carotene intake caused a decrease in milk fat vitamin A ester, carotene and xanthophyll levels. When, however, the feed was supplemented during period 7*b* with 0·75 g. carotene in oil to give a total carotene intake of approximately 0·85 g./day, milk fat vitamin A ester and carotene values rose rapidly, the vitamin A ester levels for a short period exceeding those in the milk fat of the control cows. By the end of period 7*b* vitamin A ester levels were identical in the control and treated cows and no significant changes occurred on return to full pasture during the post-experimental period. Carotene, on the other hand, was still slightly lower in the milk fat of the treated cows than of the controls at the end of period 7*b* and a slight increase occurred on return to full pasture. Xanthophyll levels returned rapidly to normal during the post-experimental period.

Milk and fat yields remained relatively constant throughout the two experiments, and no significant differences were found between treated and control cows.

DISCUSSION

As noted previously (10), a problem inherent in this type of experiment in New Zealand is the relatively short time during which cows, normally pastured fed throughout the year, can be maintained on the type of dry feed necessary in a carotene-free ration. However, as also shown previously (10), milk-fat levels respond relatively quickly to changing carotene intakes and fairly steady levels of both carotene and vitamin A relative to control levels would seem to have been attained in the milk fat of the treated cows towards the end of each period.

From the results presented in Figs. 1 and 2 it is apparent that although the carotene intake of the control animals was relatively constant throughout the experiment at about 5 g./day, considerable fluctuations occurred in the levels of carotene and vitamin A in the milk fat. Thus in Fig. 1 it can be seen that milk-fat carotene varied from a maximum of 10·4 to a minimum of 8·0 μg./g., and vitamin A ester from 6·3 to 4·9 μg./g., giving a variation in total potency from approximately 40 to 30 i.u./g. That these sample-to-sample differences were of the same order as the differences between control and treated animals during period 4 (Fig. 1) would seem to indicate that some factor other than carotene intake was capable of producing as great a change in milk-fat carotene and vitamin A as a 50-fold change in carotene intake. At present we have no indication of the nature of the factor involved, but it is of interest to note throughout both experiments the close correlation between the sample-to-sample fluctuations in the milk fats of control and treated animals suggesting that variations possibly in the external environment of the animal may have a marked effect on carotene utilization.

Although these fluctuations tended to mask the effect of varying carotene intake, it appears quite clear from Fig. 1 (Exp. 1) that a relationship does exist between carotene intake and total fat potency even up to relatively high levels of carotene intake. For example, there was some slight depression of milk fat vitamin A ester levels when the carotene intake was reduced to 1·3 g./day (period 3) and milk-fat carotene levels were affected to some extent when carotene intake was reduced to 2·5 g./day (period 2). The latter effect was slight, however, so that generally speaking with intakes from pasture in excess of 2·5 g. carotene/day, it seems that the total vitamin A potency of the milk may be regarded as virtually independent of carotene intake. This is in agreement with the

earlier findings of Hibbs, Krauss & Monroe(2) and Wiseman, Sheppard & Carey(3), although in the present investigation the maximum total vitamin A potency of the milk fat produced at this level of carotene intake was lower than that reported by these workers.

Throughout the present experiments little relationship could be observed between carotene intake and the level of vitamin A alcohol in the milk fat, although there did appear to be a slight tendency for the alcohol level in the milk fat to increase as the carotene intake decreased. However, the differences noted were so small, that little or no significance can be attached to them.

In comparing the results of the second experiment (Fig. 2), in which 750 mg. carotene in arachis oil was used as almost the sole source of carotene in the diet, with those of the first experiment (Fig. 1), it is noteworthy that this level of carotene in oil was more effective than 1·3 g. of carotene from pasture in maintaining milk-fat vitamin A potency, and only slightly less effective than 2·5 g. of pasture carotene. It was also of interest that on transferring from the 'meal plus carotene in oil' rations used in Exp. 2 (period 7*b*) to full pasture, no change occurred in milk-fat vitamin A ester levels and milk-fat carotene levels increased only slightly. Carotene in oil would appear, therefore, to be considerably better utilized than carotene in pasture grown under New Zealand summer conditions.

From the results of both experiments it seems apparent that under New Zealand conditions a relationship similar to that reported by overseas workers exists between the carotene intake from pasture and the levels of vitamin A and carotene in the milk fat. During spring, autumn and winter the total vitamin A potency of the milk fat is of the same order as would be predicted from the known carotene intake of the animals, but in midsummer some factor prevents these maximum levels being attained. It would appear, further, that the limiting factors may be environmental in nature.

SUMMARY

1. The results of an investigation into the efficiency of utilization by dairy cows of carotene from New Zealand summer pasture and from arachis oil are reported.

2. A 50-fold decrease in the intake of carotene from pasture over a short period was shown to have only a small effect on the vitamin A potency of milk fat, carotene content being reduced by approximately 2μg./g. fat and vitamin A ester by approximately 1·5μg./g. fat. No change was observed in vitamin A alcohol content.

3. Sample-to-sample variations in both the control and treated animals were observed in many cases to exceed the differences due to treatment except during the period of extreme carotene deprivation. Sample-to-sample variations of control and treated animals were also observed to be highly correlated, and it is suggested that variations in the external environment of the animal may have a marked effect on carotene utilization.

4. Carotene in arachis oil was shown to be relatively more effective in maintaining the vitamin A potency of milk fat than summer pasture.

The results of this investigation form a section of a thesis submitted by one of us (N. A. W.) in partial fulfilment of the requirements for the degree of Ph.D. of the University of New Zealand. The authors are indebted to the Department of Scientific and Industrial

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684. FACTORS INFLUENCING THE VITAMIN CONTENT OF MILK FAT

VII. THE POSSIBLE EXISTENCE OF PROTEIN-BOUND CAROTENOIDS AND VITAMIN A ALCOHOL IN MAMMARY SECRETION

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In studying the levels of carotenoids and vitamin A in milk, two methods are available for the preparation of the fat for assay; it may be obtained by direct solvent extraction of the whole milk (e.g. (1)) or by separation, churning and 'oiling off' the butter (e.g. (2)). In a comparison of the carotene and vitamin A content of fats obtained from the same sample of milk by these two methods, Henry, Kon, Gillam & White (3) found that although the vitamin level was the same, there was slightly more carotene in the solvent-extracted fat. This difference they attributed to destruction of the carotene during churning. In subsequent experiments, however, Kon, Mawson & Thompson (4) suggested that the difference between the two methods of extraction lay in a relatively high concentration of carotenoids in the fraction of the original fat which remained in the separated milk. Investigating the levels of carotenoids and vitamin A in the fats obtained by solvent extraction from a range of products derived from the same samples of bulk milk, these workers showed that the concentration of carotenoids in the extracted fat increased with decreasing fat-globule size, and concluded that the carotenoids were in some way associated with the fat-globule membrane. The concentration of vitamin A, on the other hand, was found to be independent of globule size and they suggested, therefore, that it may be in true solution in the fat.

François (5) found that both carotenoids and vitamin A varied with globule size and proposed a surface layer on the fat globule highly concentrated in these substances. White, Eaton & Patton (6) extended these observations to show a positive linear correlation between globule surface/volume ratio and concentration of the carotenoids and vitamin A in the fat and derived values for the concentrations in the various portions of the fat globule. Chanda (7) has further suggested that the abnormally high levels of carotenoids previously reported (8) in the fat extracted from the milk of cows suffering from clinical mastitis might be partly explained by the decreased size, and hence increased surface/volume ratio, of the fat globules in the mastitis milk.

The work described in the present communication was commenced primarily to study the distribution of the two forms of vitamin A, alcohol and ester, between fat globules of different sizes. Previously only total vitamin A had been estimated, and it seemed possible that the conflicting results regarding its distribution might be due to differences in the alcohol to ester ratios in the milks, the more polar vitamin A alcohol tending to concentrate at the globule surface and the ester to be more uniformly distributed through the fat. During the preliminary work it became clear, however, that certain observations

were not consistent with the suggestion of a surface layer of carotenoids and vitamin A on the globule, and that the apparent change in concentration with globule size could be explained by postulating in milk, as in blood plasma (9), the presence of protein-bound forms of these substances. Further, an increased quantity of these protein-bound forms would explain the high levels of carotenoids and vitamin A alcohol in mastitis milk. Experiments were therefore carried out to investigate the presence of such protein-bound forms of carotenoids and vitamin A in normal and mastitis milk, in pre-partum secretions and in colostrum.

EXPERIMENTAL

Milk samples were collected, usually at the evening milking, from cows of The Dairy Research Institute (N.Z.) herd. Fats were prepared for analysis from these milks either by direct solvent extraction (1) or by separation, churning and 'oiling off' (2). (In this paper the former will be referred to as the 'solvent extraction' method and the latter as the 'churning' method). The method of Thompson, Ganguly & Kon (10) was used for the separation and estimation of vitamin A and carotenoids.

The first experiments were designed mainly to study the partition of vitamin A alcohol and ester between fat globules of different sizes. Since the concentration of vitamin A alcohol is extremely low in normal milk fat, three animals were dosed with 2–3 g. vitamin A* 24 hr. before the milk samples were collected. Immediately after collection portions of these samples were centrifuged at a range of speeds and times calculated to remove into the cream layer all globules larger than a desired size. Without disturbing the cream layer, as much skim-milk as possible was then withdrawn to give, from each original milk, a series of samples in which the fat was present in progressively smaller globules. Sufficient of each sample to give at least 0·5 g. fat was then solvent extracted.

In a second set of experiments, milk samples were collected separately from the four quarters of several cows suffering in varying degrees from mastitis (mainly *Streptococcus agalactiae*). Fat samples were obtained from these milks by solvent extraction. In a number of cases, since the quantity of milk was too small for normal separation and churning, fats were also obtained by centrifuging to remove into the cream layer all globules larger than about 0·5 μ and solvent extracting the cream (suitably diluted with water).

In further experiments, samples of pre-partum secretion, colostrum and normal milk were collected from individual cows. Fats were prepared from the colostrum and milk samples by the churning method. The skim-milks were centrifuged to remove into the cream layer all globules larger than about 0·2 μ. The cream layer was removed, washed twice with water and solvent extracted. Pre-partum samples were too small for normal separation, and were therefore diluted with water and centrifuged as above.

Protein fractions were then prepared from the centrifuged serum samples as follows:

Casein by precipitation with dilute acetic acid at pH 4·6. The precipitate was washed by dissolving in dilute ammonia and reprecipitating twice. In some cases a small amount of fatty material separated during washing. This was centrifuged off, solvent extracted and referred to as 'casein fat'.

* The vitamin A used was a proprietary emulsion containing approximately 8 mg. vitamin A/g.

*Globulin and albumin** by precipitation at pH 6·0 with appropriate concentrations of ammonium sulphate (11) and centrifuging.

The proteins were dissolved in dilute ammonia solution and solvent extracted.

RESULTS

The effect of decreasing globule size on the concentration of carotenoids and vitamin A in the extracted fat is shown in Table 1. With decreasing globule size marked increases are apparent in the levels of carotene, xanthophyll and vitamin A alcohol. Vitamin A ester is, however, less concentrated in the fat extracted from the smaller globules. In this

Table 1. *Apparent variations in the concentrations of carotenoids and vitamin A with fat globule size (results expressed in μg./g. fat)*

Sample	Method of extraction	Vitamin A		Carotenoids		Vitamin A alcohol/ester ratio	Carotene/ xanthophyll ratio
		Alcohol	Ester	Carotene	Xanthophyll		
Whole milk	Solvent	3·9	124	13	7·8	0·31	1·67
Cream	Solvent	3·8	126	13	7·6	0·30	1·71
Butter	Churning	3·9	130	13	7·2	0·30	1·81
Fat in globules							
Less than 8 μ	Solvent	3·4	126	15	8·3	0·27	1·82
Less than 6 μ	Solvent	3·8	125	17	9·2	0·30	1·85
Less than 5 μ	Solvent	4·9	115	22	12	0·42	1·83
Less than 4 μ	Solvent	5·0	102	26	14	0·49	1·86
Less than 3 μ	Solvent	5·6	96	27	15	0·52	1·80
Less than 2 μ	Solvent	7·1	89	40	22	0·80	1·82
Less than 1 μ	Solvent	10·3	78	62	33	1·32	1·88

experiment it should be noted that the maximum globule size stated bears no direct relationship to the average globule size; that the normal distribution of globules below the size stated will have been disturbed in favour of the smaller globules by centrifuging; and that the centrifuged milk samples examined represent merely a series in which the fat is present in progressively smaller globules.

Table 2 shows the effects of mastitis on the levels of carotenoids and vitamin A in the milk fat obtained by solvent extraction and by centrifuging. In the absence of mammary gland infection there is a uniform level of carotenoids and vitamin A in the milk from the four quarters, but milks from infected quarters show, relative to normal or only slightly infected quarters, marked increases in carotene, xanthophylls and vitamin A alcohol, the magnitude of the increases being proportional to the severity of the infection. In some cases (e.g. cow 5) there is also some decrease in milk vitamin A ester. Fats obtained from mastitis milk by solvent extraction were considerably higher in carotene, xanthophyll and vitamin A alcohol than the corresponding fats obtained by centrifuging.

The distribution of carotenoids and vitamin A between fat and protein fractions in pre-partum secretions, colostrum and milk is shown in Table 3. In all cases the bulk of the carotenoids and vitamin A was found to be associated with the fat but in the pre-partum secretions, and to a progressively lesser extent in the colostrum and milk samples, some carotene, xanthophyll and vitamin A alcohol was associated with the various

* The term globulin as used in this paper refers to the mixture of euglobulin, pseudo globulin and immune globulins precipitated at about 58% saturation with ammonium sulphate (2·3 M). Albumin refers to the mixture of β-lactoglobulin and α-lactalbumin precipitated by increasing the concentration of ammonium sulphate to about 80% saturation (3·3 M).

Table 2. *Effect of mastitis on the levels of carotenoids in the milk fat of cows (results expressed in μg./g. fat)*

Cow no.	Quarter	Degree of infection*	Vitamin A		Carotenoids		Vitamin A alcohol/ester ratio	Carotene/ xanthophyll ratio
			Alcohol	Ester	Carotene	Xanthophyll		
			Solvent extracted fats					
1	LF	0	0·20	5·6	8·6	1·8	0·036	4·8
	LH	0	0·22	5·7	8·5	1·7	0·039	5·0
	RF	0	0·19	5·5	8·3	1·7	0·035	4·9
	RH	0	0·20	5·7	8·9	1·8	0·034	4·9
2	LF	0	0·34	6·3	18	2·3	0·054	7·8
	LH	0	0·34	6·5	17	2·4	0·052	7·1
	RF	2	0·47	6·2	19	2·5	0·076	7·6
	RH	5	0·84	6·4	28	3·5	0·13	8·0
3	LF	1	0·58	6·8	15	2·8	0·085	5·4
	LH	1	0·71	7·1	16	2·9	0·100	5·5
	RF	3	1·62	7·2	26	4·1	0·231	6·3
	RH	1	0·60	7·0	15	2·8	0·086	5·4
4	LF	5	1·75	8·4	30	6·7	0·21	4·5
	LH	2	1·05	8·9	26	5·7	0·12	4·6
	RF	1	0·59	8·4	16	4·5	0·070	3·6
	RH	1	0·71	8·7	17	4·0	0·082	4·2
5	LF	10+	4·3	4·6	670	110	0·94	6·1
	LH	6	1·51	6·8	43	19	0·22	2·2
	RF	3	0·73	6·2	24	12	0·12	2·0
	RH	10+	3·3	4·1	420	78	0·81	5·4
			Centrifuged fats					
1	All	0	0·20	5·7	8·4	1·7	0·035	4·9
4	LF	5	1·02	8·5	21	4·8	0·12	4·4
	RH	1	0·58	8·8	14	3·2	0·066	4·4
2	RH	5	0·53	6·8	18	3·2	0·078	5·6
3	RF	3						
5	LF and RH	10+	1·1	4·8	89	21	0·23	4·2
Normal milk. Solvent extracted			0·15	5·5	10·8	3·5	0·027	3·1
Normal milk + 10% plasma. Solvent extracted			1·20	6·1	110	3·4	0·20	32
Normal milk + 10% plasma. Churned			0·17	6·0	12·5	3·1	0·028	4·0

* An empirical scale from 0 to 10 indicating approximate degree of infection.

protein fractions, particularly the globulin. Although the fat was removed as completely as possible before protein precipitation, extraction of the various protein fractions frequently yielded traces of fat. In calculating the quantities of carotenoids and vitamin A associated with the various protein fractions, allowance has been made, where necessary, for this fat, the assumption being made, in line with arguments advanced later in this communication, that the concentrations in this fat were the same as in the main fat fraction. All such corrections were, however, extremely small and generally negligible. It is of interest to note that if, as is usual in the solvent extraction method, the carotenoids extracted from some of the protein fractions had been expressed as concentrations in the traces of fat extracted, extremely high values would have been recorded. The highest concentration of carotene, for example, expressed on this basis, was 3500 μg./g. fat, and in a number of cases, the levels were over 1000 μg./g. fat.

The 'fat fraction' shown in Table 3 represents the sum of the fat samples obtained by churning and by centrifuging together with any fat which separates during protein fractionation. In a number of cases these samples were assayed separately and the

Table 3. *Distribution of carotenoids and vitamin A between fat and protein in pre-partum secretion, colostrum and milk (results expressed in μg./100 ml. and percentage distribution)*

Fraction	Vitamin A: Alcohol (μg.)	Vitamin A: Alcohol (%)	Vitamin A: Ester (μg.)	Vitamin A: Ester (%)	Carotenoids: Carotene (μg.)	Carotenoids: Carotene (%)	Carotenoids: Xanthophyll (μg.)	Carotenoids: Xanthophyll (%)	Carotene/xanthophyll ratio
Pre-partum secretion (average 3 samples)									
Fat	1·30	48	17	94	93	62	3·8	88	24
Casein	0·35	13	0·3	2	7·5	5	—	—	—
Globulin	0·74	27	0·5	3	44	30	0·5	12	88
Albumin	0·31	12	0·2	1	4·2	3	—	—	—
	2·70	100	18·0	100	149	100	4·3	100	
Colostrum (average 3 samples)									
Fat	0·86	68	86	98	160	95	46	98	3·5
Casein	0·20	16	2	2	1·3	1	0·3	1	4·3
Globulin	0·21	16	—	—	7·0	4	0·4	1	18
Albumin	—	—	—	—	0·2	—	—	—	—
	1·27	100	88	100	169	100	47	100	
Milk (average 3 samples)									
Fat	0·6	87	22	100	43	97	14	97	3·1
Casein	0·02	3	—	—	0·3	1	0·1	1	3·0
Globulin	0·06	9	—	—	0·8	2	0·1	1	8·0
Albumin	0·01	1	—	—	0·1	—	0·1	1	1·0
	0·69	100	22	100	44·2	100	14·3	100	

Table 4. *Distribution of carotenoids and vitamin A in various samples of fat obtained from the colostrum and milk of individual cows (μg./g. fat)*

Cow	Fat	Vitamin A: Alcohol	Vitamin A: Ester	Carotenoids: Carotene	Carotenoids: Xanthophyll	Carotene/xanthophyll ratio
		Colostrum				
A	Churned	0·30	30	55	16	3·4
	Centrifuged from skim-milk	0·55	10	63	20	3·1
B	Churned	0·14	14	35	1·6	22
	Centrifuged from skim-milk	0·20	5·6	40	1·6	25
	'Casein fat'	0·22	2·7	22	1·0	22
		Milk				
C	Churned	0·25	6·8	11·5	2·8	4·1
	Centrifuged from skim-milk	0·27	5·9	12·9	2·9	4·5
	Skim-milk solvent extracted	0·52	4·6	21·8	3·9	5·6
D*	Churned	3·6	57	11·8	5·6	2·1
	Centrifuged from skim-milk	3·6	34	10·3	4·6	2·2

* Cow dosed 3 g. vitamin A 24 hr. previously.

results from several individual colostrum and milk samples are shown in Table 4. It is apparent that there is a decreased concentration of vitamin A ester in the fat derived from the smaller globules, but that the levels of the other constituents are in general relatively independent of globule size. Thus, the variation in carotene and vitamin A alcohol concentration is only apparent when the fat is obtained by solvent extraction.

DISCUSSION

The results presented here support previous observations (4,5,6) that the fat obtained by solvent extraction from smaller globules of milk fat is more concentrated in carotenoids than fat obtained from the larger globules present in the same milk sample. These observations are consistent with the theory, advanced by Kon *et al.* (4), that there is present on the fat-globule surface a layer concentrated in carotenoids. On analogy with other fat-globule constituents it seemed reasonable to assume that in addition to the fraction in true solution in the triglyceride fat, some of the carotenoids and vitamin A were associated with the fat-globule membrane, and King (12) has assigned a place to vitamin A at least in his postulated structure of the globule membrane. There is, however, some evidence against such a surface concentration of carotenoids and vitamin A. In the first place, the fat in buttermilk is rich in membrane material and would be expected to contain high concentrations of any substances associated with the globule membrane. However, Kon *et al.* (4) found that fat extracted from buttermilk was little different in carotenoid and vitamin A content from churned fat. Furthermore, in the present investigation, the levels of carotenoids and vitamin A were fairly uniform in the different fat fractions obtained by churning or centrifuging (Table 4) despite the differences in the sizes of the globules from which the fats were obtained.

Clearly the carotenoids and vitamin A in milk do not exist entirely in simple solution in the triglycerides of the fat globules, but there is no direct evidence to show that there is any concentration of these substances at the globule surface. The results presented here would seem to indicate rather the presence, in mammary secretions, of carotenoids and vitamin A in association with serum proteins and independent of the fat globules. Such protein-bound materials would be extracted with the fat, and in experiments such as those described in Table 1, and as reported by Kon *et al.* (4), would have the effect of increasing the concentrations in fat obtained by solvent extraction from milk and its products. This increase in concentration in the extracted fat would be progressively more apparent as the size of the globules, and hence the quantity of fat, relative to volume of milk, decreased.

During milk synthesis there is evidence of the uptake by the mammary gland, among other blood constituents, of chylomicrons and plasma proteins (e.g. review by Glascock, (13)). It has been suggested previously (14) that under normal conditions of adequate carotene intake, the bulk of the carotenoids and vitamin A in milk are derived through this uptake of chylomicrons containing vitamin A ester and carotenoids of immediate dietary origin. At the same time there is evidence of the uptake from the blood of some vitamin A alcohol and carotenoids not of immediate dietary origin. In blood these forms of vitamin A alcohol and carotenoids have been shown to exist in close association with plasma proteins, probably mainly globulins (e.g. review by Deuel (9)), and it would seem reasonable to assume that they are taken up by the mammary gland along with the plasma proteins (again mainly the globulins since an increased level of globulin, as, for example, in colostrum and in mastitis milk, is always associated with increased levels of carotenoids and vitamin A).

During the changes which occur in the cells of the mammary gland much of this vitamin A alcohol and carotenoids must become dissociated from the proteins and be transferred to the fat, the vitamin A alcohol being fairly completely esterified in the

process (14,15). This change from protein association to fat phase is apparent in the case of cows on a diet devoid of carotene or vitamin A. Here the protein-bound vitamin A alcohol and carotenoids of the blood provide the only source of milk carotenoids and vitamin A, but these substances are found, as in normal milk, mainly in the fat globules (14). From the results presented in this paper, however, it is suggested that in all milks a small proportion of the vitamin A alcohol and carotenoids remains associated with the globulins (some components of the globulin fraction may be merely transudates of blood) or becomes associated with other milk serum proteins during the course of synthesis in the mammary gland. Where the uptake of blood proteins is higher than usual, more of the vitamin A alcohol and carotenoids remain associated with the proteins. This is exemplified by the results presented in Table 3. Although in all the samples investigated, the bulk of the carotenoids and vitamin A was associated with the fat, some carotene, xanthophylls and vitamin A alcohol was precipitated from virtually fat-free serum with the different proteins, particularly the globulin fraction. The proportion of protein-bound material was highest in the pre-partum secretions and decreased through the colostral period to normal milk, where only relatively small amounts were present. There was little or no evidence of any vitamin A ester associated with protein. It is of interest to note, in Table 3, the marked differences in the composition of the carotenoids present in the fat and globulin fractions, and also the transition in carotenoid composition from pre-partum secretion to normal milk. Blood carotenoids contain a higher proportion of carotene than milk-fat carotenoids, and the ratios shown would further support the suggestion that the milk-globulin carotenoids derive directly from blood-protein carotenoids, and that the carotenoids in the fat at the start of lactation are also derived largely from the same source.

From the results presented in Table 2 it appears that, although protein precipitation was not carried out, mastitis milk is similar, in the carotenoid and vitamin A content and distribution, to pre-partum secretions or to colostrum, depending on the severity of the infection. Thus, although the vitamin A alcohol and carotenoid content of the fat obtained by centrifuging milk from infected quarters was higher than normal, it was still considerably lower than that found in comparable solvent-extracted fat, indicating the probable presence in the mastitis milk of increased amounts of protein-bound vitamin A alcohol and carotenoids. A similarity in composition between mastitis milk and colostral secretions would also be anticipated from a consideration of the pathological changes in the udder associated with mastitis (16). The results of an isolated experiment, also recorded in Table 2, show that, as would be expected, the addition of blood plasma to normal milk produced typical 'mastitis' levels of vitamin A alcohol and carotenoids in the fat obtained by solvent extraction. The fat obtained by churning was more normal, but it is apparent that either on standing or during separation and churning, a small amount of blood carotene had become associated with the milk fat.

From the results presented in Tables 1 and 4 it would appear that the concentration of vitamin A ester in the milk fat decreases with decreasing globule size. Some such decrease might be expected in the concentration of any constituent in solution in the triglyceride portion of the fat, since the proportion of triglyceride in the extracted fat will decrease slightly with decreasing globule size (17). This decrease is, however, considered to be too small to explain the large differences in vitamin A ester particularly in Table 1 and this aspect of the work is being further investigated.

SUMMARY

1. The distribution of carotenoids and vitamin A in milk-fat globules has been further investigated. The results obtained support previous observations that the carotenoid content of the fat obtained by solvent extraction from the smaller globules in milk is greater than in the fat obtained from the larger globules. It has also been shown that the vitamin A alcohol content of the extracted fat varies in the same way as the carotenoid content, but that the reverse appears to be the case for vitamin A ester.

2. It is suggested that these differences in concentration are not due to a surface layer of carotenoids and vitamin A alcohol on the fat globule but to the presence in mammary secretions, as in blood, of protein-bound forms of these substances. In support of this it has been shown that when fat is obtained by centrifuging rather than by solvent extraction, its carotenoid and vitamin A content appears to be almost independent of globule size. Furthermore, some vitamin A and carotenoids remained in the virtually fat-free serum obtained from normal milk, colostrum and pre-partum secretion and was precipitated with the various serum proteins, particularly the globulin fraction. The increased carotenoid and vitamin A alcohol content of mastitis milk may also be explained by the presence of abnormally large amounts of these protein-bound forms.

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685. FACTORS INFLUENCING THE DESTRUCTION BY HEAT OF VITAMIN B_{12} IN MILK

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Much of the vitamin B_{12} of liquid milk may be destroyed during processing. Thus, sterilization 'in-bottle' may cause a loss of more than 90%, and the preparation of 'evaporated' milk about 80% loss (Chapman, Ford, Kon, Rowland, Thompson, Crossley & Rothsell, 1956)(1). This paper describes an investigation of means whereby this destruction of the vitamin can be reduced.

EXPERIMENTAL

Methods. Vitamin B_{12} was assayed microbiologically with *Lactobacillus leichmannii*, as described by Gregory, 1954(2).

Ascorbic acid was determined by reaction with 2:6-dichlorophenolindophenol, as described by Harris & Olliver (1942)(3). Total ascorbic acid, defined as the sum of ascorbic acid, dehydroascorbic acid, and 2:3-diketogulonic acid, was measured by the dinitrophenylhydrazine method of Roe & Kuether, (1943)(4), modified as recommended by Geschwind, Williams & Li (1951)(5).

Thiamine was determined microbiologically with *Lb. fermenti*, as described by Sarett & Cheldelin (1944)(6).

Except where otherwise indicated, the milk used was bulk morning milk from the Institute mixed herd of Friesian and Shorthorn cows. In-bottle sterilization was carried out in amber-coloured glass mineral-water bottles, of 105 ml. absolute capacity, containing 100 ml. portions of milk. The bottles were sealed with 'Crown' corks and heated in a steam autoclave—in general to 110° C. for 20 min.

RESULTS

Experiment 1. A first empirical approach was to examine the effects of selected compounds added to the milk before heating. Two such compounds were sodium cyanide and potassium metabisulphite, both of which are used to stabilize vitamin B_{12} during the processes of microbiological assay (cf. Ford, 1953(7); Krieger, 1952(8)). The effect of SH compounds (e.g. glutathione, cysteine and thioglycollate) was examined to test the possibility that the destruction of vitamin B_{12} might be related to the increase in SH titre on heating.

A variety of antioxidants and inhibitors of protein denaturation (sucrose and sodium dodecyl sulphate) were tested. Thiamine and ascorbic acid were examined, as being labile and potentially reactive native constituents of the milk. Furthermore, it has been shown that vitamin B_{12} is unstable to heating with a large molar excess of ascorbic acid, particularly in the presence of copper (Rosenberg, 1956)(9). The results are set out in Table 1.

The loss of vitamin B_{12} was markedly increased by the added SH compounds and ascorbic acid, and prevented by the addition of cyanide or bisulphite, and to a lesser

extent by EDTA and thiosulphate. No one of the last four compounds can be suggested as a practical means of stabilizing vitamin B_{12} in commercial milk, even were such additions to milk legally permissible: cyanide for obvious reasons; bisulphite because in the concentration effective in stabilizing vitamin B_{12} it accentuates the cooked flavour and greatly increases the destruction of thiamine. It was observed, however, that besides protecting vitamin B_{12}, both cyanide and bisulphite largely prevented the heat loss of ascorbic acid. EDTA, on the other hand, while reducing the destruction of vitamin B_{12}, greatly accelerated the loss of ascorbic acid. Thiosulphate is relatively ineffective in protecting vitamin B_{12}. Its influence on the heat stability of thiamine and ascorbic acid was not investigated.

Table 1

Supplement added to milk before heating	Percentage loss of vitamin B_{12} on heating
None	67
Sodium cyanide, 0·2 mg./100 ml.	20
Sodium cyanide, 2 mg./100 ml.	0
Glutathione (reduced), 10 mg./100 ml.	> 90
Cysteine hydrochloride, 10 mg./100 ml.	> 90
Thioglycollic acid, 10 mg./100 ml.	> 90*
Thiourea, 10 mg./100 ml.	60
Sodium thiosulphate hydrochloride, 40 mg./100 ml.	45
Potassium metabisulphite, 5 mg./100 ml.	50
Potassium metabisulphite, 25 mg./100 ml.	0*
Ascorbic acid, 5 mg./100 ml.	80
Ethylenediamine tetra-acetic acid, 10 mg./100 ml.	20
Sodium nitrite, 10 mg./100 ml.	55
Thiamine hydrochloride, 10 mg./100 ml.	70
Gallic acid, 10 mg./100 ml.	75
Butylated anisole, 10 mg./100 ml.	70
Tetraethylthiuram disulphide, 10 mg./100 ml.	85
Sodium dodecyl sulphate, 10 mg./100 ml.	70
Sucrose, 5 g./100 ml.	65

* At the concentration indicated, both these compounds prevented browning of the milk on heating.

Influence of dissolved oxygen on the heat stability of vitamins in milk

It seemed possible that the destruction of vitamin B_{12} in heated milk might be associated, directly or indirectly, with the oxidative degradation of ascorbic acid (cf. Rosenberg, 1956 (9)). It in turn is known to be linked with the concentration of oxygen dissolved in the milk (Kon & Watson, 1936 (10), Sharp, Hand & Gurthrie, 1939 (11)), and it was therefore pertinent to examine the influence of dissolved oxygen on the stability of vitamin B_{12}.

Experiment 2. Milk from one cow was collected through a teat syphon and 'Polythene' tube into a brown glass vessel of 2 l. capacity and containing a layer of medicinal paraffin. Six 100 ml. samples of the milk were then bottled, with minimal exposure to air. Two of the filled bottles were heated at 110° C. for 20 min., two at 120° C for 30 min., and two were kept as unheated 'controls'. The remainder of the milk was aerated by vigorous stirring for 3 min. and filled into six further bottles, which were treated as described above. The effects of the various treatments on vitamin B_{12} and ascorbic acid ('total', measured by the method of Roe & Kuether (4)) are shown in Table 2.

The destruction of vitamin B_{12} was much greater in the aerated milk, particularly during sterilization at the higher temperature. The finding with the 'protected' milk that loss of vitamin B_{12} was no higher under the more drastic conditions of sterilization argues against purely thermal destruction, or simple interaction with some native constituent

of the milk, and suggests that the vitamin in fact may be a casualty of a side reaction on the pathway of the breakdown of ascorbic acid.

Table 2. *Effect of aeration on the stability of vitamin* B_{12} *and ascorbic acid in milk towards heating*

Sample	Vitamin B_{12} (mμg./ml.)	Ascorbic acid (μg./ml.)
Raw milk, protected from exposure to air	2·9	14
Same, bottled and heated to 110° C. for 20 min.	1·4	16
Same, but heated to 120° C. for 30 min.	1·4	Not measured
Raw milk, aerated	2·7	15
Same, bottled and heated to 110° C. for 20 min.	0·63	5
Same, but heated to 120° C. for 30 min.	0·2	Not measured

Experiment 3. A 1 gal. sample of bulk morning milk was divided into two portions. Six 100 ml. samples of one portion were bottled. Two of the bottles were heated at 110° C. for 20 min., two at 120° C. for 30 min. and two were kept as unheated controls. The second portion was held under vacuum at 45° C. and flushed with a slow stream of nitrogen for 15 min. Six 100 ml. portions of this deoxygenated milk were then bottled, with minimal exposure to the air. The bottles were gassed with nitrogen during filling, to the moment of sealing the stoppers. In this way the air in the head space was diluted with nitrogen. The bottles were then treated as described above. The effects of the different treatments on vitamin B_{12}, thiamine and ascorbic acid are shown in Table 3.

Table 3. *Effects of removal of oxygen on the thermal destruction of vitamin* B_{12}, *ascorbic acid and thiamine in milk*

Sample	Vitamin B_{12} (mμg./ml.)	Ascorbic acid (μg./ml.)*	Thiamine (μg./ml.)
Bulk milk; unheated	4·2	30	0·50
Bulk milk, sterilized in bottle at 110° C. for 20 min.	1·2	13	0·34
Bulk milk, sterilized in bottle at 120° C. for 30 min.	0·8	Not measured	0·27
Nitrogen-gassed bulk milk; unheated	4·2	33	0·50
Nitrogen-gassed bulk milk; sterilized in bottle at 110° C. for 20 min.	3·8	29	0·28
Nitrogen-gassed bulk milk sterilized in bottle at 120° C. for 30 min.	3·6	Not measured	0·22

* By indophenol titration. The values for the unheated milks and the sterilized, nitrogen-gassed milk are somewhat higher than are normally found.

These findings again show that the extent of the destruction of vitamin B_{12}, as of ascorbic acid, during in-bottle sterilization is determined to a large extent by the degree of aeration of the milk. Destruction of thiamine, on the other hand, was not reduced by deaeration of the milk before heating. In this, and in one other experiment, the indications were that the loss on heating was slightly greater in the deaerated milk.

The efficacy of deaeration as a means of stabilizing vitamin B_{12} varied between different samples of bulk milk. Losses on heating ranged from 0 to 50%; but in all instances the destruction of vitamin B_{12} was very much greater in milk that had not been deaerated.

In the next experiment, the influence of ascorbic acid, dehydroascorbic acid, glutathione and oxygen was examined in relation to the stability towards heating of vitamin B_{12} in solution.

Experiment 4. Portions of a solution of vitamin B_{12} in phosphate buffer were bottled and sterilized in the presence or in the absence of oxygen, and with various combinations of ascorbic acid, dehydroascorbic acid and reduced glutathione.

To 3 l. of 0·02 M phosphate buffer of pH 6·7 were added 15 μg. vitamin B_{12}. The solution, in a large wash-bottle, was warmed to 50° C. in a water-bath, and gassed for 1 hr. with nitrogen. One-half of the solution was then transferred to another wash-bottle and gassed with oxygen for 30 min., during which period gassing of the remaining solution with nitrogen was continued. Meanwhile, to pairs of 100 ml. brown glass bottles were added 1 ml. portions of nitrogen-gassed buffer containing in solution (1) 3 mg. ascorbic acid or (2) 3 mg. dehydroascorbic acid,* or (3) 3 mg. reduced glutathione, or (4) 3 mg. ascorbic acid and 3 mg. reduced glutathione, or (5) 3 mg. dehydroascorbic acid and 3 mg. reduced glutathione, or (6) no further addition. One of each pair of bottles was then filled to within 1 ml. of capacity with the nitrogen-gassed buffer solution and the other with the oxygen-gassed solution. The bottles were stoppered and heated in a steam autoclave for 20 min. at 110° C. After cooling, the contents were assayed for ascorbic acid and vitamin B_{12}. The results are shown in Table 4.

Table 4. *Factors affecting the thermal destruction of vitamin* B_{12} *and ascorbic acid in solution in phosphate buffer of* pH 6·7

Compounds added* with vitamin B_{12} (0·005 μg./ml.) to the buffer	Nitrogen-gassed buffer. Percentage destruction of			Oxygen-gassed buffer. Percentage destruction of		
	Vitamin B_{12}	Ascorbic acid (indophenol test)	Ascorbic acid (Roe & Kuether test)	Vitamin B_{12}	Ascorbic acid (indophenol test	Ascorbic acid (Roe & Kuether test)
None	19 (24)†	—	—	18 (25)	—	—
Glutathione	31 (71)	—	—	26 (40)	—	—
Ascorbic acid	17 (44)	65	65 (100)	50 (94)	100	100 (100)
Dehydroascorbic acid	26 (81)	—	100 (100)	32 (65)	—	100 (100)
Ascorbic acid and glutathione	18 (29)	50	50 (100)	29 (46)	100	100 (100)
Dehydroascorbic acid and glutathione	22 (51)	—	100 (100)	25 (37)	—	100 (100)

* Each at 30 μg./ml.

† The figures in parentheses were obtained in another experiment, in which the test solutions were heated more severely to 240° C. for 30 min.

In the buffer gassed with oxygen, both ascorbic acid and the preparation of dehydroascorbic acid increased the destruction of vitamin B_{12}, whereas in an atmosphere of nitrogen only the dehydroascorbic acid caused appreciable loss. It would seem that ascorbic acid itself can be excluded as a specific destructive agent. At the same time, there is no implication that dehydroascorbic acid rather than ascorbic acid was involved, since in the crude preparation of dehydroascorbic acid used there were doubtless present other products of the oxidation of ascorbic acid.

Reduced glutathione increased the destruction of vitamin B_{12}, more particularly in the absence of oxygen. Yet, on the other hand, it largely neutralized the destructive influence of ascorbic acid in the presence of oxygen.

DISCUSSION

The mechanism whereby vitamin B_{12} is destroyed during the heat treatment of milk is not clear, though some of the present findings suggest that the process is essentially oxidative. The addition of ascorbic acid or glutathione to milk or to a solution of

* A crude preparation, made by treating a solution of ascorbic acid with active charcoal.

vitamin B_{12} in phosphate buffer enhanced the destruction of vitamin B_{12} caused by heating, and one suspects that any theory as to the mechanism whereby this destruction is brought about should be applicable to both substances. There are in fact suggestions in the literature of certain similarities in the oxidation of these two compounds. The oxidation of ascorbic acid by copper to dehydroascorbic acid has been reported to liberate hydrogen peroxide (Barron, De Meio & Klemperer, 1935–36(12); Dekker & Dickinson, 1940(13)) which then appears to form a product with ascorbic acid that can bring about the hydroxylation of a wide variety of aromatic compounds (Udenfriend, Clark, Axelrod & Brodie, 1954)(14). Similarly, it has been postulated (Young & Young, 1942)(15) that hydrogen peroxide is also produced when glutathione is oxidized with copper. Hydrogen peroxide has been reported to inactivate vitamin B_{12} (Beiler, Moss & Martin, 1951)(16). The ability of cyanide and bisulphite to stabilize vitamin B_{12} could perhaps be explained in terms of their effect in maintaining ascorbic acid and glutathione in their reduced forms.

This interpretation is complicated by the somewhat paradoxical observation that the destructive influence of ascorbic acid was diminished in the presence of glutathione. It may be suggested that the vicarious oxidation of glutathione that occurs in the presence of ascorbic acid involves a different mechanism, but it seems better to defer further speculation on this point until we have more precise knowledge of the manner in which vitamin B_{12} is degraded.

In fresh milk glutathione appears to have only a fleeting existence, if indeed it occurs at all, and when added to milk it is very rapidly destroyed (Gould, 1940)(17). However, other sulphydryl compounds are exposed or liberated by the heat denaturation of the milk proteins, and it seems possible that the destruction of vitamin B_{12} could be a consequence of their oxidation. Thus, cysteine increased the destruction of vitamin B_{12} in milk during heating; and cysteine is readily oxidized by copper ions (Rosenthal & Voegtlin, 1933)(18).

The results obtained in these experiments indicate that ascorbic acid, or sulphydryl compounds liberated by denaturation of milk protein, or glutathione, may each or all be involved in the destruction of vitamin B_{12} that occurs when milk is heated. The removal of dissolved oxygen may offer a practical means of preserving the vitamin, as well as of stabilizing the ascorbic acid and preventing the development of oxidized flavour (Sharp *et al.* 1939)(11).

SUMMARY

The processing of liquid milk, particularly sterilization 'in-bottle' or 'in-can', may cause considerable destruction of its content of vitamin B_{12}. This loss can be greatly reduced by thorough deaeration of the milk before heating.

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686. THE FATTY ACIDS OF BUTTERFAT AND THE VOLATILE ACIDS FORMED ON OXIDATION

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It has been long established that C_4–C_{12} volatile fatty acids with an even number of carbon atoms normally occur to the extent of approximately 6% by weight in the glycerides of milk fats(1). Recent studies, using gas-liquid chromatography have shown that volatile fatty acids with an odd number of carbon atoms form part of the small amount of volatile fatty acid present in the glycerides of some animal fats(2,3,4). It would be reasonable to expect, therefore, that the same sensitive techniques would reveal the presence of these acids in butterfat, and the present experiments were carried out with this object. However, this investigation has failed to bring to light any new fatty acid components of butterfat.

Since Fritsch & Deatherage(5) found that autoxidation of 9:10 monoene compounds gave rise to formic, acetic, and propionic acids, it was thought that the odd-numbered volatile fatty acids reported in natural fats might also be derived from autoxidative changes which might have occurred during the preparation of the fats for analysis. To test this hypothesis autoxidized butterfat was examined and shown to contain, in contrast to fresh butterfat, formic, propionic and nonanoic acids. Butterfat is a convenient raw material for work of this nature, because it can be obtained in pure form without solvent extraction and prolonged evaporation of solvents.

EXPERIMENTAL

The butterfat used in these experiments was representative of the bulk churning of cream from numerous outdoor pasture-fed herds (mainly of the Jersey breed).

Preparation of steam volatile fatty acid for analysis

The butterfat (usually 2–2·5 g. samples) was saponified with concentrated aqueous sodium hydroxide. The soaps were transferred to a micro-distillation apparatus with the minimum volume of water, acidified with sulphuric acid and steam distilled until the titratable distillate was negligible. The volatile acids were titrated and a small excess of alkali added before evaporation of the soaps *in vacuo* on a rotary evaporator at approximately 60° C. The soaps were concentrated to a known volume and an aliquot evaporated to dryness in an extraction vessel(6). Sodium-dried and distilled ether was added to the dry soaps, which were then acidified with saturated $KHSO_4$ solution. Anhydrous Na_2SO_4 was added, the mixture thoroughly ground and the ether decanted into a 5 ml. flask. Not less than six extractions were made with small volumes of ether until the total extracts measured 5 ml.

Separation of steam volatile acids by gas-liquid chromatography

The 4 ft. columns packed into a tube of 4 mm. internal diameter, consisted of Celite 545 (Johns Manville and Co. Ltd.) (5 parts), with silicone MS550 (Hopkin & Williams Ltd.) (2 parts), behenic acid (0·2 parts), and *o*-phosphoric acid (0·2 parts) as the liquid phase (7). A bubbler containing water was placed in the nitrogen stream between the manostat and the chromatogram. This column packing overcame the necessity for the operation of the column at 100° C. for the separation of the lower acids. At low flow rates (10–12 ml. N_2/min.), separations of formic and acetic acids were possible at 137° C. so that this temperature was used for separating all acids between C_1 and C_{12}.

An appropriate volume of the ether solution of the fatty acids was injected into the chromatographic column with an 'Agla' micrometer syringe. The ether was evaporated by passing nitrogen through the chromatogram at room temperature. When all the ether was evaporated, the nitrogen flow was stopped and the chromatogram brought to operating temperature. Subsequent operation was carried out as described by James & Martin (8).

Separation of methyl esters of the non-volatile fatty acids by gas-liquid chromatography

Methyl esters prepared from the non-volatile fatty acids by treatment with 1% H_2SO_4 in methanol (1) were separated at 206° C. on 5 ft. columns of 4 mm. internal diameter packed with Celite 545 (5 parts) and Apiezon M vacuum stopcock grease (Shell Chemicals Ltd.) (1 part). The esters were detected and estimated as they emerged from the chromatogram by thermal conductivity measurement (9) with a coiled platinum filament (Gow Mac Instrument Co.). The carrier gas (hydrogen) was passed through an identical reference cell before the point of sample injection. The two cells were connected to a Wheatstone bridge circuit which was supplied by a 6 V. accumulator. Changes in the temperature and hence the resistance of the detector cell were recorded on a Phillips Recorder with a 1 mV. span.

Samples were introduced through a self-sealing rubber cap by means of an 'Agla' micrometer syringe fitted with a 0·5 mm. bore stainless steel needle.

The unsaturated fatty acids were identified by chromatographing the esters after hydrogenation. Hydrogenation was carried out at 170° C. and at atmospheric pressure with Raney Nickel as catalyst.

Autoxidation of butterfat

Aeration of butterfat or its Na soaps was carried out by passing air at the rate of 100 ml./min. in a fine stream through 2 g. samples held at 35° C. or at 70° C. A small amount of Antifoam A (Dow Corning Corp.) was added to the Na soaps to prevent foaming. Aeration was continued for 6 hr. at the rate of 100 ml./min. in all cases. Volatile material of an acidic nature formed during aeration was trapped in dilute sodium hydroxide solution.

The autoxidized samples and the volatile material trapped in dilute sodium hydroxide during autoxidation were prepared for analysis by gas-liquid chromatography as described above.

RESULTS

No volatile fatty acids with an odd number of carbon atoms have been detected in freshly prepared butterfat. The composition of the steam volatile fatty acids with an even number of carbon atoms is given in Table 1, accompanied by the composition of the non-volatile acids, as determined by gas-liquid chromatography of the methyl esters.

Table 1. *The composition (moles %) of the fatty acids of butterfat*

	Method of analysis		
	Gas-liquid chromatography		Esterfractionation Hansen & Shorland[10] (Sept. sample)
Acid	(*a*)	(*b*)	
Saturated			
C_2	0·28	—	—
C_4	9·08	—	11·5
C_6	3·92	—	4·5
C_8	1·84	Trace	1·5
C_{10}	1·33	1·40	3·5
C_{12}	0·17	2·86	3·5
C_{14}	—	9·55	9·6
C_{16}	—	23·30	21·6
C_{18}	—	13·85	11·3
C_{20}	—	0·96	0·7
Unsaturated			
C_{10}	—	—	0·2
C_{12}	—	—	0·2
C_{14}	—	2·00	0·8
C_{16}	—	1·66	1·8
C_{18}	—	26·60	28·1
C_{20}	—	1·22	1·3

(*a*) Separated as the acids. (*b*) Separated as the methyl esters.

The steam distillation procedure appears to give quantitative recoveries of acids up to C_8, but C_{10} was distributed evenly between the volatile and non-volatile fractions. Neither did steam distillation give a clear-cut fractionation of C_{12} acid. The volatile fatty acids (C_2–C_{10}) present in butterfat, were of the order of 16·5 mol. %.

On the other hand, analyses of the same butterfat after mild autoxidation revealed the presence of steam volatile fatty acids, with an odd number of carbon atoms in amounts which varied with the conditions of autoxidation (Table 2).

Table 2. *The volatile fatty acids (moles %) of oxidized butterfat*

	Conditions of autoxidation*			
	Glyceride oxidation			Na salt oxidation
		70° C.		
Acid	35° C.	Non-volatile	Volatile	70° C.
Formic	—	0·5	0·2	0·9
Acetic	2·1	1·4	0·3	1·3
Propionic	—	—	0·1	0·3
Butyric	57·8	53·1	0·1	53·4
Valeric	0·4	—	—	0·7
Hexanoic	22·6	23·4	—	22·9
Octanoic	9·7	9·4	—	10·6
Nonanoic	0·4	1·9	—	—
Decanoic	7·0	9·6	—	9·9

* Samples were aerated at the rate of 100 ml. air/min. for 6 hr.

As there are considerable amounts of the volatile acids with even carbon number in the control samples of butterfat, a comparison of the amounts in these and autoxidized samples did not give adequate evidence that autoxidation gave rise to additional even-numbered acids of short-chain lengths. However, the presence of acetic and butyric acids along with formic and propionic acids in the volatile material collected during the autoxidation of glycerides of butterfat points to such a conclusion.

Formic acid was not found to be a natural constituent acid of butterfat, but Table 2 shows that significant amounts appeared when both the glycerides of butterfat and the derived soaps were oxidized at 70° C. Autoxidation of glycerides produced nonanoic acid at both 35 and 70° C., but this acid was absent when the Na soaps were autoxidized. In the latter case, however, a wide range of steam volatile acids with odd carbon numbers appeared, the amount of formic acid being particularly large. Formic acid was not found when glycerides were autoxidized at 35° C.

The analysis of the fatty acids of butterfat provided an opportunity for comparing the present method with the ester fractionation technique. The analysis by the latter method was also for New Zealand butterfat (September sample)(10). There is good agreement between the two sets of figures.

Unsaturated fatty esters were identified as the components which were present in the unhydrogenated sample, but were absent in the hydrogenated sample. In the particular stationary phase used on the chromatogram the unsaturated esters ran ahead of the saturated esters with the same carbon number. The proportions of odd-numbered acids and branched-chain acids in butterfat(11) are too small to be detected by the thermal conductivity cell.

DISCUSSION

In the present experiments no evidence has been obtained for the presence of volatile fatty acids with odd carbon numbers in freshly prepared butterfat. Recently some of these acids, namely, valeric and heptanoic acids, were found in goat-milk fat(4), but they were not found in cow-milk or cow-udder fat(12). Neither have the odd acids been found in ewe-milk fat(13). It remains to be seen whether this represents a difference in the composition of goat-milk fat as compared with the fat of cow and ewe milk, or whether it is due to secondary processes.

By analogy with animal tissue fats it was expected that the odd acids would be present in butterfat. For example, mutton fat(2) and ox perinephric fat(3) contain 0·01–0·06% of volatile fatty acids by weight, of which considerable proportions (up to 42·5%) consisted of acids with odd carbon numbers. Fairbairn(14) also found odd acids (valeric and a trace of propionic) in the female reproductive organs of *Ascaris lumbricoides*, which is a rich source of volatile fatty acids (12·9% by weight).

Numerous investigators who have carried out autoxidation experiments on fats, found that volatile fatty acids were among the products of autoxidation and of these acids some had odd carbon numbers. For example, the autoxidation of oleic acid at 100° C. gave rise to traces of formic and acetic acids(15), the autoxidation of elaidic acid at 47–78° C. produced formic acid(16) and the autoxidation of methyl oleate at 80° C. yielded predominantly formic acid, some acetic acid and, occasionally, traces of propionic acid(5). In this latter work Fritsch & Deatherage found that small amounts of the volatile fatty acids were detected as soon as peroxide formation occurred, indicating that fragmentation

occurred in the relatively early stages of autoxidation. They postulated that formic and other acids could be formed from the breakdown of hydroxyalkyl radical, which is formed from the hydroxyalkyl peroxide.

In the light of these and the present findings it might be expected that the naturally occurring volatile acids could easily be contaminated with acids arising from autoxidation of fat during its preparation for analysis. The present experiments have shown that volatile acids with odd carbon numbers could not be detected in control samples, whereas the odd acids, particularly formic, propionic and nonanoic acids are found in butterfat which had been subjected to mild conditions of autoxidation for relatively short periods of time.

The formation of volatile acids during the autoxidation of the sodium salts of the acids is particularly interesting in that conditions which could lead to autoxidation exist during the removal of ethanol, following the saponification of fats prior to fatty acid analysis (1). In large samples of fat, removal of ethanol at this stage is difficult, and it involves heating for long periods of time at approximately 90° C. At a certain stage in the removal of ethanol, the vacuum has to be broken repeatedly to avoid excessive foaming. These conditions could be expected to produce certain short-chain acids. In the present experiments saponification of the glycerides was carried out with aqueous alkali which, although a little less efficient, might be preferable in this type of investigation to using ethanolic alkali.

The extent to which volatile acids produced by autoxidative changes as distinct from the volatile acids produced by lipolytic enzymes of micro-organism (17) contribute to storage flavours in butter has not been assessed. However, acids from the former source are likely to be partly responsible for the 'off' flavour in stored butter, because, as mentioned above, Fritsch & Deatherage (5) found that the volatile acids are among the products of autoxidation from methyl oleate in the early stages of peroxide formation.

SUMMARY

1. Despite the presence of appreciable quantities of steam volatile fatty acids as glycerides in butterfat, no short-chain fatty acids with odd carbon number have been detected by gas-liquid chromatography of the acids.

2. Mild oxidation of butterfat for short periods of time produces steam volatile fatty acids with odd carbon number. A large proportion of these acids was formic, with smaller proportions of propionic, valeric and nonanoic acids.

3. The relative ease with which short-chain fatty acids are produced on oxidation of fats, points to the danger of contaminating naturally occurring fatty acids with extraneous acids.

4. A total analysis of the fatty acids of New Zealand butterfat by gas-liquid chromatography compared well with similar analyses carried out by previous workers by means of esterfractionation analysis.

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687. CHANGES IN SIZE OF FAT GLOBULES IN CREAM DURING VACREATOR* TREATMENT

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(With 2 Figures)

Pasteurization of cream with direct steam in a vacuum pasteurizer usually leads to higher fat losses in churning than does pasteurization by indirect heating even where dilution of cream with condensed steam is avoided. The increased losses have been attributed to disruption of fat globules during the vacuum pasteurization process (McDowall(1)). Sargent(2) found that the average diameter of fat globules in cream was slightly increased by flash pasteurization and definitely reduced by Vacreator treatment of the cream. More detailed measurements by Dolby(3) showed that flash pasteurization caused a decrease in the number of small globules and a marked increase in the number of large globules in the cream. Vacreator treatment caused an increase in the proportions of both small and large globules, but the clumping effect was much less pronounced than with flash pasteurization.

Sargent (*loc. cit.*) observed a progressive reduction in globule size in the two units of the Vacreator. The average diameter of globules found as a mean of the two experiments was 2·53 μ for the raw cream and 2·17, 1·85 and 1·58 μ for samples from the pasteurizing section, deodorizer section and water cooler, respectively.

No further studies on changes in fat globules brought about in individual sections of the Vacreator have been reported. It has been suggested that disruption of globules is caused principally by a slight homogenizing effect in the passage of cream past the bomb valve (McDowall(1)). The flash-boiling which takes place in each stage when the cream enters a region of higher vacuum may also have an influence on the size of the globules. The present investigation was planned to study these questions in more detail.

OPERATING PRINCIPLES OF THE VACREATOR

A simplified diagram of a Tandem Vacreator is given in Fig. 1. Cream enters the pasteurizing section through a float valve and falls in drops from the perforated bottom of a spray pan. Steam is admitted at the top of the chamber and raises the cream to pasteurizing temperature (say 204° F.). A mixture of steam and cream is drawn through the equilibrium valve (E.V.) and uptake pipe to the separating chamber. A vacuum of 10–15 in. is maintained in this chamber by the ejector pump controlled by an air admission valve at the top of the chamber. The setting of the E.V. determines the vacuum drop between the pasteurizing chamber and the uptake pipe and hence fixes the pasteurizing temperature. Additional steam for deodorizing purposes may be introduced into the uptake pipe through the infuser valve (I.V.).

* 'Vacreator' is the registered trade mark of the Murray Vacuum Pasteurizer.

Cream gravitates from the first separating chamber to the intermediate float tank (I.F.T.) whence it is drawn via an uptake pipe to a second separating chamber held at about 28 in. of vacuum. It is extracted from this chamber by a centrifugal pump and passed to the cooler.

Sampling of cream from various stages of the Vacreator is possible through drain-cocks at the points *A*, *B*, *C* and *D*.

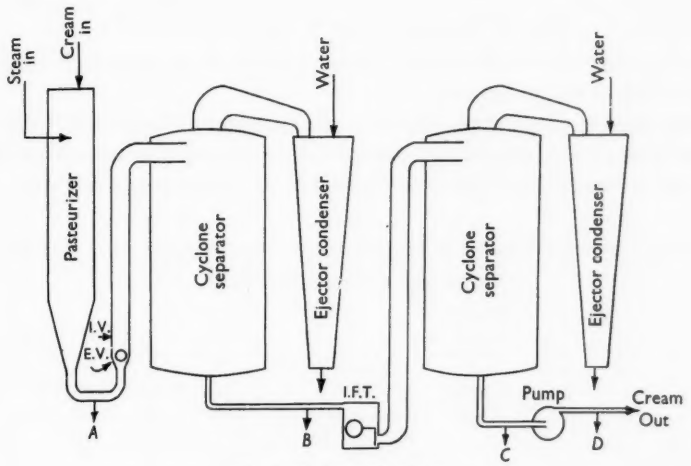

Fig. 1. Tandem Vacraetor.

EXPERIMENTAL

Factory methods

Experimental treatment of cream was done in a Baby Tandem Vacreator. Unless otherwise stated, the cream used was sweet cream with a pH of about 6·7 and a fat content of 37–40%. Samples were taken from the drain-cocks by attaching a bottle to the cock with a rubber bung. When the cock was opened cream collected slowly in the bottle. When a sufficient volume had accumulated, the cock was closed and the bottle removed. In order to flush the drain-cock a quantity was collected and discarded before the actual sample was taken. Samples were immediately cooled under running water to about 70° F.

Laboratory methods

Microscopic method. Preparations were made and globules measured as described in an earlier paper (3), with the following modifications:

Preliminary standing of diluted cream to remove 'oiled-off fat' was omitted. To improve the accuracy of estimation of the larger globules, additional measurements were made of globules over 5μ in diameter. Usually all the globules in four fields on each of two preparations were measured. The larger globules ($>5\mu$) were measured in an additional ten or twelve fields on each preparation. These additional measurements were used to correct the numbers of globules of over 6μ diameter.

All results were converted from numerical percentages of globules of each size to distribution percentages, i.e. proportion of total fat present as globules of each size. For

presentation in the tables, the results have been further condensed into groups, e.g. fat as globules 0·5, 1·0 and 1·5 μ in diameter has been shown as '$<2\mu$'.

Rapid method. In some of the later experiments estimations of the proportions of fat present as small globules were made by the centrifugal method recently described (4).

RESULTS

Effect of successive stages of vacreator treatment

In Table 1 are summarized the results of four experiments in which samples were taken at successive points in the machine.

In all four runs the cream at the bottom of the pasteurizer chamber contained more fat as small globules ($<2\mu$) than did the original cream. A greater increase in small globules had occurred in cream which had passsed the E.V. and separating chamber.

Table 1. *Size distribution of fat globules in cream samples taken at different stages of Vacreator treatment*

Exp. no.	Vacuum 1st unit (in.)	Vacuum 2nd unit (in.)	Intensity (lb. steam /lb. cream)	Sampling point	Fat in globule size class as percentage total fat $<2\mu$	$2–3\mu$	$4–6\mu$	$7–10\mu$	$>10\mu$
1·1 (6. xi. 52)	13	27	0·40	Raw cream	1·4	14	50	14	20
				Past. sect. (*A*)	2·2	13	39	26	19
				I.F.T. (*B*)	3·9	20	46	24	7
				Pump inlet (*C*)	1·0	5	18	18	59
				Pump outlet (*D*)	1·0	5	17	22	55
1·2 (15. xii. 52)	13	28	0·35	Raw cream	1·4	13	56	29	0
				Past. sect. (*A*)	2·4	16	50	29	2
				I.F.T. (*B*)	3·3	21	47	21	7
				Pump inlet (*C*)	1·8	11	40	35	11
				Pump outlet (*D*)	3·0	14	46	30	6
1·3 (16. iii. 53)	13	28	0·39	Raw cream	2·0	19	52	22	5
				Past. sect. (*A*)	2·8	17	38	23	18
				I.F.T. (*B*)	4·2	19	39	24	16
				Pump inlet (*C*)	2·4	11	30	37	21
				Pump outlet (*D*)	2·8	13	37	22	26
1·4 (31. viii. 53)	13	28	0·41	Raw cream	1·3	10	43	41	5
				I.F.T. (*B*)	2·7	14	39	32	13
				Pump outlet (*D*)	1·0	4	15	35	45

In the second or cooler unit of the Vacreator a marked reduction in small globules and increase in large globules ($>10\mu$) took place. Passage through the extractor pump caused (in two out of three experiments) a further subdivision of globules.

The disruption of globules occurring in the stage including the E.V. was expected, but it was not anticipated that subdivision of globules would also occur in the pasteurizing chamber.

Pasteurizing chamber

Experiments were made to determine whether the disruption of globules at this stage was due to the sudden raising of temperature of cream droplets on meeting the steam or to violent turbulence produced by steam admission.

In two experiments a comparison was made of low and high rates of steam flow. The results (see Table 2, Exps. 2·1, 2·2) showed that at a low intensity no subdivision of

globules took place, although in Exp. 2·1 some clumping (formation of globules over 10 μ diameter) occurred. At high intensity both splitting and clumping of globules took place.

Table 2. *Effect of pasteurizer section on size distribution of fat globules*

Exp. no.	Sampling point	Steam inlet	Lb. steam /lb. cream	Fat in globule size class as percentage total fat					Serum fat / Total fat
				$< 2\mu$	2–3 μ	4–6 μ	7–10 μ	$> 10\mu$	
2·1	Raw cream	—	—	2·2	19	59	20	—	—
	Past. sect. (*A*)	Original	0·21	1·5	14	42	14	28	—
	Past. sect. (*A*)	Original	0·59	2·5	15	35	17	30	—
2·2	Raw cream	—	—	1·8	18	50	23	7	—
	Past. sect. (*A*)	Original	0·17	1·9	20	53	22	3	—
	Past. sect. (*A*)	Original	0·67	2·3	13	31	27	27	—
2·3	Raw cream	—	—	1·2	9	31	27	31	1·3
	Past. sect. (*A*)	Original	0·40	1·4	10	35	33	21	2·3
	Past. sect. (*A*)	Modified	0·40	1·2	9	39	41	10	1·8
2·4	Raw cream	—	—	1·0	10	49	32	8	0·9
	Past. sect. (*A*)	Original	0·36	1·9	12	41	32	13	1·2
	Past. sect. (*A*)	Modified	0·36	1·3	11	42	35	11	1·1

It was concluded that the damage to globules in this section was due to the high velocity of the incoming steam producing sufficient turbulence to have some homogenizing effect.

To test this theory a new steam inlet, designed to reduce the velocity of steam entering the chamber, was constructed. This inlet was built into a short additional section which could be inserted in the pasteurizing chamber (see Fig. 2). The lower section (*D*) contained the original inlet in which steam was admitted tangentially without expansion. The section (*C*) containing the modified inlet was mounted between the float valve section (*A*) and the pasteurizing chamber proper (*D*). The new inlet was of conical shape and was connected radially. With this arrangement it was possible to make a direct comparison of the effect of the two inlets. When using the new inlet, the sections were clamped together in the order shown in Fig. 2. To use the old inlet the spray pan (*B*) was placed in its original position in the top of the pasteurizing chamber (*D*).

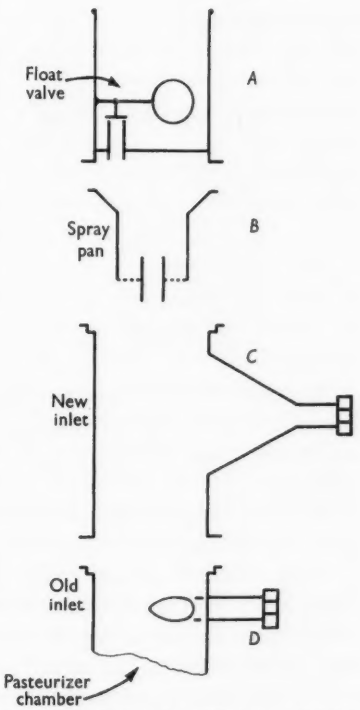

Fig. 2. Original and modified steam inlets.

The results in Table 2, Exps. 2·3 and 2·4, showed that the modified inlet caused much less splitting of globules than the original inlet. The microscopic results were confirmed by estimations of fat in centrifuge serum from the diluted cream.

Equilibrium valve

The effect of removing the E.V. is shown in Exp. 3·1 (Table 3). (With the E.V. out, the pasteurizing temperature is, of course, lowered nearly to the value corresponding to the vacuum in the first separating chamber—179° F. for 15 in.) Samples were taken from the outlet of the extractor pump (point *D*, Fig. 1). With the E.V. removed, the outgoing cream contained only slightly more fat as small globules than did the original cream, but it did contain more large globules. This clumping could have occurred in the second unit of the Vacreator and not at the site of the E.V.

Table 3. *Effect of vacuum drop across equilibrium valve on size distribution of fat globules in vacreator cream*

Exp. no.	Sampling point	Conditions	Vacuum 1st unit (in.)	Vacuum 2nd unit (in.)	Fat in globule size class as percentage total fat $<2\mu$	$2–3\mu$	$4–6\mu$	$7–10\mu$	$>10\mu$	Serum fat / Total fat
3·1	Raw cream	—	—	—	1·6	12	45	37	4	—
	Pump outlet (*D*)	E.V. in	15	26	3·4	11	34	40	12	—
	Pump outlet (*D*)	E.V. out	15	26	1·8	7	25	42	24	—
3·2	Raw cream	—	—	—	1·4	22	58	13	6	—
	Pump outlet (*D*)	E.V. and I.F.V. in	15	22	2·1	16	37	37	8	—
	Pump outlet (*D*)	E.V. and I.F.V. in	10	13	1·4	13	37	20	28	—
	Pump outlet (*D*)	E.V. and I.F.V. out	10	13	1·6	10	27	29	33	—
3·3	Raw cream	—	—	—	1·2	15	56	23	5	1·7
	I.F.T. (*B*)	—	4	28	1·5	7	21	35	36	1·6
	I.F.T. (*B*)	—	17	28	3·6	17	32	27	20	3·9
	Pump outlet (*D*)	—	4	28	—	—	—	—	—	1·8
	Pump outlet (*D*)	—	17	28	—	—	—	—	—	2·6
3·4	Raw cream	—	—	—	1·8	17	45	27	10	1·3
	I.F.T. (*B*)	—	4	15	—	—	—	—	—	1·4
	I.F.T. (*B*)	—	15	28	—	—	—	—	—	2·2
	Pump outlet (*D*)	—	4	15	1·3	10	32	29	28	1·5
	Pump outlet (*D*)	—	15	28	1·7	8	21	37	32	1·9

Removal of both E.V. and intermediate float valve (I.F.V.) so that there was only a small vacuum drop throughout the machine reduced splitting, but not clumping of globules and was no more effective than simply lowering the vacuum on the first unit (see Exp. 3·2, Table 3).

In further experiments on reduction of vacuum in the first unit, sampling before the I.F.T. showed more clearly the changes taking place in each of the two units.

With a vacuum of only 4 in. in the first unit (Exp. 3·3, Table 3) splitting of globules was greatly reduced, but some clumping occurred. Passage through the second unit at full vacuum did not appreciably increase the numbers of small globules as shown by the fat content of centrifuge serum.

Use of a low vacuum in both units (4 and 15 in., respectively) (Exp. 3·4, Table 3) kept splitting of globules to a minimum, but there was still some clumping of globules.

Use of infuser

Introduction of a part of the deodorizing steam through the infuser instead of into the pasteurizer reduces the flow of steam through the E.V., and so may be expected to lessen

the disruption of globules at this point. This expectation was confirmed in two experiments (Table 4). In the first (Exp. 4·1) sufficient steam to give a rise of 10° F. in the condenser water was put through the pasteurizer. The infuser valve was then opened enough to increase the rise by a further 50° F. Operation in this manner resulted in considerably less splitting of globules than operation with the same steam flow all going through the pasteurizer.

Table 4. *Effect of use of infuser on size distribution of fat globules in vacreator-treated cream*

Exp. no.	Sampling point	Lb. steam/lb. cream: Pasteurizer	Lb. steam/lb. cream: Infuser	Fat in globule size class as percentage total fat: $< 2\mu$	$2–3\mu$	$4–6\mu$	$7–10\mu$	$> 10\mu$	Serum fat / Total fat
4·1	Raw cream	—	—	—	—	—	—	—	1·1
	I.F.T. (*B*)	0·50	—	3·6	21	51	17	7	2·2
	I.F.T (*B*)	0·08	0·42	2·5	19	49	22	8	1·6
4·2	Raw cream	—	—	—	—	—	—	—	0·7
	I.F.T. (*B*)	0·50	—	—	—	—	—	—	2·3
	I.F.T. (*B*)	0·25	0·25	—	—	—	—	—	1·9
	Pump outlet (*D*)	0·50	—	—	—	—	—	—	2·2
	Pump outlet (*D*)	0·25	0·25	—	—	—	—	—	2·0

In a second experiment (Exp. 4·2) it was shown that diversion of 50% of the treatment steam to the infuser also gave appreciably less splitting of globules. The creams from the two runs in this experiment were churned separately under similar conditions. The percentages of fat lost in the buttermilk were 1·67 for the control and 1·47 with use of I.V.

If too large a proportion of the steam used is taken through the I.V. the time of contact between steam and cream is reduced and deodorization efficiency is lowered. According to McDowall(5) up to 50% of the steam may be put through the infuser without lowering efficiency.

Extractor pump

Data given in Table 1 showed that the extractor pump may cause some disruption of globules, but that the effect is variable. Further experiments gave similar results. On some occasions the size distribution of globules in cream was altered very little by passage through the pump. On other occasions slight splitting or clumping of globules took place. These differences were probably caused by variations in the conditions under which the pump operated.

The extractor pump is required to remove cream from a chamber in which it is boiling under vacuum. Further boiling of cream will therefore occur at the pump inlet and in the pump itself, and the pump will have to handle a large volume of vapour unless

(*a*) the cream is cooled in passing from chamber to pump, or

(*b*) the chamber fills with cream, thus producing a gravity head between chamber and pump.

It is probable that the pump will have much less disruptive effect on the globules when it is kept full of cream than when it is pumping a mixture of cream and vapour or when cavitation occurs due to boiling of cream in the pump.

The Vacreator has provision for cooling cream by means of a water-jacket round the lower part of the separating chamber. The flow of water through this jacket, however, must be kept to a minimum or condensation of steam in the chamber will reduce deodorization efficiency and dilute the cream (McDowall(5)).

Experiments showed that passage of hot water (150° F.) through the jacket increased surging in the pump, while cold water (60° F.) reduced but did not entirely eliminate surging. The change in operating conditions did not have any clear-cut effect on extent of splitting of fat globules in the pump. It is possible that considerable momentary variations in conditions in the pump occur, as partial filling and emptying of the separating chamber alters the head at the pump inlet.

LABORATORY EXPERIMENTS

A laboratory scale investigation was made of the effect on the globules of the flash-boiling of cream which occurs when hot cream is discharged into a region at lower pressure.

Cream (approximately 40 % fat) at 160° F. was run from a separating funnel into a flask maintained at a vacuum of 29 in. The rate of inflow of cream was kept low enough to avoid any fall in vacuum. One experiment (Exp. 5·1, Table 5) showed that the boiling caused some clumping but no splitting of globules.

Table 5. *Effect of running hot cream* (160° F) *into a vacuum* (29 in.) *on size distribution of globules*

Exp. no.	Treatment	Fat in globule size class as percentage total fat				
		$< 2\mu$	$2–3\mu$	$4–6\mu$	$7–10\mu$	$> 10\mu$
5·1	Control	1·5	18	56	21	4
(8. v. 53)	Hot cream run into vacuum	1·0	10	37	36	16
5·2	Control	1·6	19	55	19	5
(18. v. 53)	Run into vacuum pH 6·5	0·9	11	41	29	18
	Run into vacuum pH 7·7	1·2	15	47	28	9

Table 6. *Effect of agitation of hot cream with and without air in turbo-emulsifier*

Date	Treatment	Fat in globule size class as percentage total fat				
		$< 2\mu$	$2–3\mu$	$4–6\mu$	$7–10\mu$	$> 10\mu$
7. vii. 53	Control	0·8	13	42	34	10
	Agitated without air	1·0	13	41	37	8
	Agitated with air	0·4	3	22	42	32

It has been observed (6) that the pH of cream has a marked effect on the splitting and clumping of globules when hot cream is agitated. A high pH (e.g. pH 8) favours splitting and a lower pH (e.g. pH 6) facilitates clumping. To find whether the effect of flash-boiling was influenced by pH, two portions of cream were adjusted to pH 6·5 and 7·7, respectively. The creams were heated and run into an evacuated flask as in Exp. 5·1. The results (Table 5, Exp. 5·2) showed that clumping was more pronounced at the lower pH, but that even at the higher pH splitting was not evident.

The effect of presence or absence of air during agitation of hot cream was also investigated. For this experiment a turbo-emulsifier was used. This instrument has a rotor carrying vanes which throw the liquid against a series of fixed pins carried on a ring surrounding the rotor. One portion of the cream was treated in an open beaker so that air was beaten into the cream. Another portion was agitated for the same time and at the same speed in a beaker closed with a bung which touched the surface of the cream so that

no air was enclosed. In the latter run very little change in globule sizes was observed. Agitation in the presence of air, on the other hand, caused extensive clumping and a reduction in the number of small globules.

Other experiments already reported (6) have shown that more violent agitation in the presence of air (e.g. in a Waring Blendor) can cause splitting as well as clumping of globules. It is probable that the presence of a vapour phase (steam) will have the same effect as air.

DISCUSSION AND CONCLUSIONS

Laboratory experiments have indicated that flash-boiling of cream can cause clumping but not splitting of globules. Violent agitation of hot cream in the presence of air or vapour can cause both clumping and splitting.

Experiments with the Vacreator have shown that wherever a mixture of steam and cream attains a high velocity, splitting and clumping of globules takes place. Changes in design or operation of the plant to reduce the velocity lessen the amount of splitting of globules which occurs. These conclusions should apply with any pasteurizing or deodorizing equipment using direct steam.

In the Vacreator, splitting of globules in the pasteurizing vessel can be reduced by arranging for the velocity of the incoming steam to be lessened before the steam meets the drops of cream falling from the spray pan.

Disruption of globules at the E.V. can be lessened by reducing the vacuum difference across the valve or by introducing part of the steam at the I.V.

The flash-boiling which occurs in the cooler unit of the Vacreator results in clumping rather than splitting of globules and so counteracts some of the disruption which has taken place in the previous stage.

The extractor pump may cause a slight further splitting of globules. The disruptive effect of the pump is likely to be less where the pump is kept full of cream than where it has to handle both cream and vapour. The provision of a cooler to reduce the temperature of cream entering the pump would thus be an advantage. This cooler should be placed below the separating chamber so that it will not cause condensation of steam in the chamber.

SUMMARY

The size distribution of fat globules was determined microscopically in cream samples taken from different parts of the Vacreator during treatment of cream.

A considerable increase in numbers of small globules (splitting) was found in samples which had passed the equilibrium valve. Smaller increases took place in the pasteurizing chamber and in the extractor pump. Increase in numbers of large globules (clumping) occurred in the cooler section.

Splitting of globules could be reduced: in the pasteurizer by reducing velocity of incoming steam; at the equilibrium valve by reducing the vacuum drop across the valve or by introducing part of the steam at the infuser instead of into the pasteurizer.

It is concluded that splitting of globules in the Vacreator is due to a homogenizing effect where steam and cream travel at a high velocity. Flash-boiling causes clumping but not splitting of globules. These conclusions should apply to any equipment for pasteurization or deodorization of cream with direct steam.

The thanks of the author are due to Mr J. Singleton (Superintendent) and Mr B. Le Heron (Buttermaker) of the Institute factory for arranging and carrying out Vacreator treatments of cream, to Dr F. H. McDowall for helpful advice and criticism and to Mrs R. P. Haverkamp for assistance in measuring fat globules.

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688. PHAGE-ORGANISM RELATIONSHIPS AMONG STRAINS OF *STREPTOCOCCUS CREMORIS*: THE SELECTION OF STRAINS AS CHEESE STARTERS

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The relationships between races of bacteriophage and strains of bacteria have been used as a means of classification of organisms in epidemiological investigations particularly with salmonella strains (Craigie & Yen (1), Felix & Callow (2)) and with staphylococci (Fisk (3), Wilson & Atkinson (4), Williams & Rippon (5)). The application of a series of test-phage races to the bacterial strains isolated during the course of an epidemic and determination of lytic patterns has resulted in a differentiation more precise than that possible by other methods.

The same principle has now been applied for a considerable time in the selection of strains of *Streptococcus cremoris* to be used as starters in the manufacture of cheese. The emphasis here, however, is not upon the detection of similarities between strains, which may indicate a common origin, but upon the isolation of as many unrelated strains as possible. The use each day of a cheese starter with a different lytic pattern from the starters used on the previous 2 or 3 days considerably reduces the risk of air-borne phage infection, which is a major hazard during the maintenance of the starter culture under commercial conditions.

In the earlier stages of our work Hunter (6) found that the phages which lyse strains of *Str. lactis* are in general distinct from those which attack strains of *Str. cremoris*. Moreover, the lactis phages are usually polyvalent, whereas cremoris phages show a tendency to strain specificity. Where they are not specific to one strain their lytic activity is at least limited to a group of a few strains with a variation in titre on the different strains within the group. Nicholas & Hoyle (7), in a comprehensive investigation covering both lactis and cremoris types, made an attempt to divide the strains into groups each showing a characteristic lytic pattern with a set of type phages. Our work has been devoted mainly to the practical end of providing for commercial cheese factories a series of strains of *Str. cremoris* unrelated in their phage reactions. In the course of the work a small collection of bacterial strains and the phages which lyse them has been built up and maintained for many years (the earliest isolations were made in 1935). Purification of both cultures and phages has been carried out several times each year. The size of the collection is limited by the facilities available (it is much more limited in numbers than the collection investigated by Nicholas & Hoyle), and this fact must be borne in mind in the drawing of any general conclusions from the present data about the relationships between bacterial strains and phages, since it is very possible that the 'cross-section' observed is not large enough. With this reservation in mind it is still useful perhaps to draw attention to some features of the relationships which exist between a series of nineteen cremoris strains and a similar number of phage races.

METHODS

The bacterial cultures were maintained in daily subculture in sterilized skim-milk at 22° C. with replating at two-monthly intervals on lactose yeast phosphate (L.Y.P.) agar (Hunter (8)) and purification by selection of a colony yielding a culture with maximum acid-producing activity. The phage races, isolated in the first instance from cheese whey were purified by isolation of single plaques on a bacterial mat on L.Y.P. agar. High titre preparations were made by lysis of the susceptible strains growing in sterilized skim-milk. When lysis was complete the milk protein was precipitated with lactic acid and after filtration through a sterilized paper filter the clear fluid was neutralized and finally Seitz-filtered. The clear fluid with a phage titre of the order of 10^{-7} as determined on an agar plate was stored in the refrigerator at 5° C. The phages were labelled according to the strain on which they were prepared, but this must not be taken to mean that any phage has necessarily a unique relationship with any one strain. For instance phage hp is not necessarily the only race which acts on strain HP and phage hp may act upon other strains. All the phages used are virulent races. Lysogenesis has not been observed in cultures of *Str. cremoris* in New Zealand, although lysogenic strains of lactic streptococci have been reported in Europe (9) and Australia (10).

The tests for lytic action were carried out by placing drops (taken by loop) of a series of decimal dilutions of a phage preparation on a mat of each of the bacterial strains on the surface of an agar plate. The results were read after overnight incubation at 30° C. Wherever a phage acted on more than one of the strains it always gave the maximum titre on the strain on which it had been prepared and usually (but not invariably) a lower titre on the other strains. In recording the results the notation of Williams & Rippon (5) was used, viz. 5 indicates maximum titre, e.g. 10^{-7}; 4 indicates 1/10th–1/100 maximum titre, e.g. 10^{-6}–10^{-5}; 3 indicates 1/1000–1/10,000 maximum titre, e.g. 10^{-4}–10^{-3}; 2 indicates 1/100,000–1/million maximum titre, e.g. 10^{-2}–10^{-1}; – indicates no action of any kind.

The results are given in Table 1 in which the strains and phages are arranged in an order which gives grouping together of those showing the closest relationship to one another. The symbols other than figures in Table 1 indicate relationships involving adaptation or inhibition.

Adaptation

Where a phage acted on more than one strain, but to different titres on the several strains, it was found, in accordance with general experience, that propagation of the phage on one of the low-titre strains yielded a phage preparation which now showed its maximum titre on this strain, and often (but not always) a lower titre on the original strain. Tests on L.Y.P. agar did not disclose all the lytic relationships which existed. Wherever a negative result was obtained on a plate a further test was made by adding to a 9 ml. tube of sterilized skim-milk 2 drops (0·1 ml.) of a 24 hr. milk culture of the strain and 1 ml. of a high-titre phage preparation. After incubation for 5 hr. at 30° C., supernatant fluid from the culture was diluted serially and spotted on a mat of the same strain on agar. By this method it was possible in some cases to obtain plaques which could be used to produce high-titre phage preparations on the strain against which no action on agar could originally be demonstrated. Sometimes a second subculture in milk of the mixture of bacterial strain and phage was necessary before plaques could be obtained on an agar plate. It appeared, therefore, that there were different degrees of relationship between

phages and strains. The closer degrees were made clear by frank lysis although perhaps to a lower than maximum titre. The more distant degrees were made evident only by treatment of a culture in a liquid medium with a massive dose of a high-titre phage. These adaptations may be either mutations or host-controlled modifications of phage (Collins (11), Anderson & Fraser (12)). We have not had the opportunity to carry out the work necessary to decide this question. Instances where the more distant type of relationship leading to adaptation were found to exist are indicated by letter A in Table 1.

Table 1. *Action of phage races on strains of* Str. cremoris.

Bacterial strain	Phage races																	
	hp	op_4	tem_2	rw	yp_7	r_6	k	fh	ud_5	am_1	hs_2	e_8	d_9	tr	d_4	ml_2	r_1	ml_1	kh
HP	5	4	4	—	—	—	—	—	—	—	—	—	—	—	I.S.	—	—	—	—
OP_4	3	5	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
TEM_2	4	A	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
RW	—	—	—	5	5	5	4	—	—	—	—	—	—	—	—	—	—	—	—
YP_7	—	—	—	5	5	5	5	—	—	—	—	—	—	—	—	—	—	—	—
R_6	—	—	—	I.L.	4	5	—	—	—	—	—	—	—	—	I.S.	—	—	—	—
K	—	—	—	I.L.	I.L.	I.L.	5	—	—	—	—	—	—	—	I.S.	—	—	—	—
FH	—	—	—	—	—	—	—	5	4	3	3	I.L.	4	A	I.S.	—	—	—	—
UD_5	—	—	—	—	—	—	—	3	5	2	3	I.L.	5	3	I.L.	—	—	—	—
AM_1	—	—	—	—	—	—	—	—	—	5	4	5	3	2	I.S.	—	—	—	—
HS_2	—	—	—	—	—	—	—	A	—	3	5	3	3	A	I.S.	—	—	—	—
E_8	—	—	—	—	—	—	—	A	—	5	5	5	2	—	I.L.	—	—	—	—
D_9	—	—	—	—	—	—	—	I.L.	I.S.	I.L.	I.L.	I.L.	5	5	4	—	—	—	—
TR	—	—	—	—	—	—	—	I.L.	—	I.L.	I.L.	I.L.	3	5	4	—	—	—	—
D_4	—	—	—	—	—	—	—	I.L.	—	I.L.	I.L.	—	4	5	5	—	—	—	—
ML_2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	5	—	—	—
R_1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	I.S.	—	5	—	—
ML_1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—
KH	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	5

5, max. titre; 4, 10^{-1}–10^{-2}; 3, 10^{-3}–10^{-4}; 2, 10^{-5} or more of max. titre; A, adaptation; I.S. inhibition on solid medium; I.L., inhibition in liquid medium.

To illustrate this adaptive change in phage which leads to changes in host range, high-titre tr phage, which undergoes adaptation to attack cultures FH and HS_2 (indicated in Table 1 by the letter A), was allowed to act on these cultures for 5 hr. at 30° C. Supernatant fluids from the mixtures, when spotted on mats of FH and HS_2, yielded plaques from which phages were prepared by action on FH and HS_2. These phage preparations, tested against all cultures on which the original tr phage had acted, gave the results indicated in Table 2. Original reactions of tr are repeated from Table 1 to facilitate comparison.

It will be seen from Table 2 that the process of adaptation resulted in a change of the range of action and in some cases of the type of action of the phage. In each case a phage preparation showed its most potent action on the culture on which it was prepared.

Inhibition

On the agar plates used for determination of lytic reactions there was in some instances evidence of an inhibitory action of a phage on a bacterial strain. In these cases the first one or two dilutions of phage produced a clear area which appeared to indicate confluent lysis, but no plaques appeared in the areas where small drops of higher dilutions had been

placed. Tests in which a 9 ml. culture in skim-milk was subjected to the action of 1 ml. of high-titre phage gave confirmation of these instances of inhibition and also disclosed further examples of lesser degrees of inhibition not sufficiently marked to show on the surface of a plate. In liquid culture the inhibition was estimated by comparison of the extent of growth, observable under the microscope, in control and phage-containing tubes after incubation at 30° C. for 5 hr. The inhibition reactions were shown to be due to the

Table 2. *Influence of adaptation on host range*

Bacterial strain	tr	tr adapted to FH	tr adapted to HS_2
FH	A	5	I.L.
UD_5	3	A	A
AM_1	2	A	5
HS_2	A	A	5
E_8	—	—	3
D_9	5	3	3
TR	5	A	2
D_4	5	A	2

phages and not to a heat-stable antibiotic produced by bacterial growth during preparation of the phages. There thus appear to exist various degrees of inhibitory action of phage races on bacterial cultures. This type of reaction, in its most marked form, is the basis of what was called by Evans (13) the nascent phage phenomenon, in which a high-titre phage formed in the presence of a second culture leads to inhibition of the second culture, giving the impression that the second culture has been lysed by a phage which does not normally attack it (Collins (14), Whitehead, East & MacIntosh (15)). The instances of inhibitory action observed in the present work are indicated in Table 1 by the letters I.S. for inhibition on a solid medium and I.L. for inhibition of a lower degree observed only in liquid medium.

Where 1 ml. of a high-titre phage failed to show any apparent influence (inhibitory or lytic) on the growth of a strain in milk and no adapted phage appeared in the culture a negative result is indicated in the Table. It is obviously possible, however, that the use of still larger proportions of phage or phage of higher titre would have made evident other relationships still more distant than those observed.

DISCUSSION

A survey of Table 1 as a whole makes it evident that many of the strains and phages tend to fall into 'family groups', and that with a few exceptions the relationships described above (involving lysis, adaptation, and inhibition) occur within these groups. The exceptions occur with phage d_4 which is distinguished from all the other cremoris phages so far isolated by its much larger particle size. It is also unrelated antigenically to the other phages. As has already been mentioned, there is a danger that the apparent position is an oversimplified picture due to the small number of strains investigated. But allowing for this possibility there still appears to be some evidence suggesting that the family grouping may mean that the strains of bacteria and races of phage forming a group have arisen in the course of time by mutation or host-controlled modification from one parent strain and one parent race.

Turning now to the practical issue it is obviously desirable that ideally there should be no relationship whatever between the members of a group of strains selected for use as starters for cheese manufacture in a day-to-day rotational system. This would usually be assured if only one strain were selected from each family group. But the ideal is difficult to attain in practice because if, as is usual, a pair of unrelated strains is used each day as an additional safeguard against phage failure, a four-day cycle means that eight unrelated cultures are required. It is very difficult to isolate a sufficient number of strains of *Str. cremoris* to meet all the requirements viz:

(1) Sufficient acid-producing activity for cheese manufacture.

(2) Sufficient heat resistance to survive cheese 'cooking' temperatures without damage.

(3) Non-susceptibility to attack by a phage race with a very high burst size or very short latent period.

(4) Absence of lytic relationship to other cultures.

(5) Absence of phage inhibitory relationship to other cultures.

(6) Absence of capacity to produce nisin-like inhibitory substances which may affect other cultures (Czulak & Hammond (16), Lightbody & Meanwell (17)).

(7) Absence of lysogenic power.

The first four properties are the most important, and taking only these into account it has been found difficult in a search extending over 10 years to assemble a group of eight strains satisfying the requirements of cheese-makers. The group of twelve strains in current use in New Zealand includes several whose activity is much lower than is desirable. It is not, therefore, possible at present to satisfy strictly requirements (5), (6) and (7) which have so far not appeared to be major sources of trouble in cheese-making practice. It is, however, desirable to keep all the requirements in mind, since a world-wide search may in time lead to the isolation of greater numbers of completely satisfactory cultures.

SUMMARY

The relationships between nineteen strains of *Str. cremoris* and nineteen phage races were investigated. It was found that in addition to clear-cut lytic reactions there were other actions between some phage races and bacterial strains whereby in some cases a phage could show adaptation to a strain and become changed in host range, and in other cases could inhibit growth of a strain without any phage multiplication.

There is some evidence to indicate that bacterial strains and phage races fall into family groups within which most of the phages act in one way or another on most of the strains. The suggestion is made that the strains and races in a group may have originated from a parent bacterial strain and parent phage race.

The selection of strains of *Str. cremoris* for use as cheese starters is discussed in the light of the findings reported in the present paper. The difficulty of isolating a large number of strains unrelated in every respect is indicated.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION B: BACTERIOLOGY AND MYCOLOGY APPLIED TO DAIRYING

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During the last 2 years bacteriology suffered many severe losses, with the death of R. S. Breed, J. M. Sherman, T. J Mackie, J. Howard Brown, Jan Kluyver and J. C. Cruickshank; men of their calibre will be sadly missed.

The last Review (1) in this series was published too late for mention of a major event in the field of new books—a new (fourth) edition of Topley & Wilson's *Principles of Bacteriology and Immunity* (2), after a lapse of 9 years; as before, a chapter is devoted to the bacteriology of milk, with special reference to public health aspects, and diseases of the udder and milk-borne diseases are also considered. In *The Life of Bacteria*, Thimann (3) deals with bacterial physiology, anatomy and biochemistry. Lees's *Biochemistry of Autotrophic Bacteria* (4) gives an excellent account of an immensely interesting subject; also Ingram in *An Introduction to the Biology of Yeasts* (5). None of the chapters in volumes 9 (1955) and 10 (1956) of *Annual Review of Microbiology* (6,7) is concerned directly with the microbiology of dairying, though many are of great interest. Likewise, the fifth and sixth Symposia of the Society for General Microbiology, published in 1955 and 1956 entitled *Mechanisms of Microbial Pathogenicity* (8) and *Bacterial Anatomy* (9); the Society has also published a useful booklet on *Constituents of Bacteriological Culture Media* (10). Of interest to all, in whatever branch of dairying, is a seventh edition of the encyclopaedic *Milchkunde und Milchhygiene* (11) by Schönberg of Hanover. Davidov's *Handbook of Dairying* (12) runs to 464 pages covering all major aspects of the Russian dairy industry, and includes a useful bibliography (147 references) of the literature on dairy products published in Russia during 1945–50; there are also many illustrations of dairy equipment and appliances in use in Russia. The new Italian journal *Giornale di Microbiologia* (13) covers a very wide field and includes English summaries. New books for the dairy bacteriologist have been few during the period under review, though some are mentioned later at the beginning of the appropriate sections.

I. LACTIC ACID AND ALLIED BACTERIA

The second edition of Seelemann's *Biologie der Streptokokken* (1) deals with the more important streptococci causing infections in man and animals, and those of significance in milk hygiene and dairying; the serological grouping of these organisms receives special attention, fourteen groups (A–P, excluding I and J) being recognized, though a chapter is devoted to biochemical techniques and Seelemann points out that the two schemes are complementary. In this edition *Streptococcus lactis* and *Str. cremoris* are placed in serological group N; it is thus encouraging to see an apparent end to the confusion that had arisen as to the priorities for groups L, M and N as a result of the restricted circulation of scientific literature during the war years. Biochemical studies of 411 strains of streptococci, representing serological groups A–M, have been made by Wahl & Meyer (2,3,4), but their collection included only a few strains of *Str. lactis*, *Str. cremoris* and *Str. durans*, and none of *Str. uberis* and *Str. thermophilus*; they indicate a preference for serological techniques for group differentiation, in suggesting that when serological tests give a clear grouping only those biochemical tests which separate the members of the individual groups need be made. Ritzerfeld (5) has also studied the biochemical properties of streptococci, using 101 strains already typed serologically, and suggests a physiological-biochemical classification with serological tests to decide doubtful cases; some of his biochemical criteria, such as haemolytic effect and 'sugar fermentations', however, are rather subject to environmental and other variation. More general papers of considerable interest from the taxonomic point of view have been published by Buchanan (6) and The Society for General Microbiology (7); the latter is, in fact, a report of a discussion meeting of the Society, being a collection of sixteen papers on various aspects of microbial classification. The work of Moreira-Jacob (8) has gone far towards a clearer definition of group E streptococci; of twenty β-haemolytic strains, sixteen of which came directly from milk, seventeen were identified as *Str. infrequens* and three as *Str. subacidus*; he found four serological types in group E, and discusses terminology within the group. Guthof (9) has described a collection of streptococci from abnormal faeces and from pus which, from his serological tests, represent a new (fifteenth) group he designates 'Q'; they differed from the enterococci also in certain biochemical and physiological characters. All of 1238 strains of streptococci from green plants and silage were identified by Schieblich & Schönherr (10), 1044 strains being *Str. faecalis*, which predominated in silage, while *Str. lactis* predominated on green maize; surprisingly, no *Str. bovis* was found.

Interest in the taxonomy of the lactic streptococci appears to have waned of late, probably because their position and intricacies are now much better understood; Sherman's (11) excellent review of these organisms is thus the more timely. He considers the status of *Str. lactis*, *Str. cremoris* and the citrate-fermenting lactic streptococci in the light of publications of the last 20 years, and much of this progress is to be seen, too, in previous Reviews of this series. Sherman discusses critically the evidence for and against *Str. lactis* and *Str. cremoris* as separate species, and *Str. diacetilactis* and *Str. citrophilus* as variants of *Str. lactis*; some (12), primarily on serological grounds (13), consider all of these, including *Str. cremoris*, to be merely variants of *Str. lactis*. The lactic streptococci, and other lactic acid bacteria, are considered elsewhwere (p. 390) from the practical standpoint, but it is interesting to note here that *Str. cremoris* has been credited with pathogenic properties, being incriminated by Brehmer (14) in a fatal case of *sepsis lenta*; though one may, perhaps, query the precise aetiology of the condition, the diagnosis of the organism was beyond reproach. In a similar case lactobacilli were isolated repeatedly from the venous blood of a nine-year-old boy suffering from subacute bacterial endocarditis; the organism was considered to be the aetiological agent in the disease but, unlike Brehmer, Dietzsch's (15) attempts to classify it into any known species were unsuccessful.

Turning to the lactobacilli, Mattick, Cheeseman, Berridge & Bottazzi (16) have developed an admirable technique for the differentiation of micro-organisms by means of paper partition chromatography; they extracted washed bacterial cells with 10% acetic acid, subjecting the extracts to two-dimensional paper chromatography, and much of the ingenuity in their work

lies in the interpretation, and methods of interpretation, of the results obtained. So far they have studied the lactobacilli and streptococci (groups B, C, D and N) in particular, and with closely related strains of species already defined physiologically and serologically, the patterns of amino acids and peptides revealed by ninhydrin were relatively constant; with different species variations were obtained, even between species hitherto not easy to differentiate. For example, the difference between *Str. lactis* and *Str. cremoris* was very marked and even that between two strains of *Lactobacillus acidophilus* which differed in their physiological reactions. Conversely, identical patterns were shown by certain cultures, originally considered distinct, subsequently shown by other methods to be, in fact, the same. *L. brevis* and *L. fermenti* could be distinguished, which is of value since the boundary between these species is not well defined. The chromatographic pictures depended to some extent on the age of the culture, the medium, and the quantity of bacteria extracted but, as the authors point out, these factors can be controlled; further development, appraisal, and practical application of this technique will be awaited with keen interest.

Dacre & Sharpe (17) have described three strains of catalase-positive *L. plantarum* isolated during work on Cheddar cheese, catalase production increasing when the strains were grown on a medium of low glucose content. Catalase-positive lactobacilli have also been encountered by Vankova (18) in studies of organisms used in Czechoslovakia for industrial lactic acid production; the strains were named *L. delbrueckii* and were similar to *Thermobacterium cereale* of Davis (19). De Man (20) has re-examined the question of the genus *Thermobacterium* (Orla-Jensen) from the standpoint of characteristics and classification, but still finds difficulties in bringing the different nomenclatures into line. Two of Vankova's strains of lactobacilli were motile, and must thus be added to those of Harrison & Hansen (21) from the turkey, Mann & Oxford (22) from the rumen, and Cunningham & Smith (23) from silage. Sharpe (24) has encountered, and overcome, the presence of haptene substances in culture media for lactobacilli, and has applied serological identification and techniques to plotting the development of *L. casei* and *L. brevis* added to starter-free cheese (25) (see Mabbitt, Chapman & Berridge (26)), using tomato juice Yeastrel Tween 80 agar (27) for isolations. Leesment (28) uses a similar medium, containing carrot extract, for the enumeration and isolation of lactobacilli from milk and milk products; for raw milk, colony representation on the medium (at pH 5·2) was lactobacilli 75%, cocci 20% and yeasts 5%. The medium was not, therefore, wholly selective for lactobacilli but, since these organisms are to a great extent morphologically distinct, the colonies could readily be enumerated and picked. The leuconostocs are a serologically diverse group of organisms and, unlike the lactobacilli, their definitive serological classification is still remote; however, Neill & Pinkes (29) have had success with some strains of *Leuconostoc mesenteroides* using antipneumococcal cross-reactions, and Jeanes, Haynes & Wilham (30) have shown that the characteristics of the dextrans produced by twenty-three strains of *Leuc. mesenteroides* isolated from sugar-cane were distinctive for each of the four colony types of these organisms; about half of the dextrans of a further fifty-five strains from other sources conformed with this grouping. The electron-microscope has been used by Gharpurey & Rao (31) in studies of the morphology of some lactic acid bacteria, from which it appears that a cell divides by internal development of a new cell wall, and not by outward constriction of the original, and with lactobacilli and streptococci they postulate a relationship between the plane of cell division and the subsequent arrangement of the groups of cells. For example, horizontal division gave rise to chain formation (*Str. faecalis* and *L. casei*), while successive divisions at right angles to the one preceding resulted in clumps (*L. brevis* and *Leuc. mesenteroides*); irregular division and even bud formation was noted with *L. acidophilus*.

In view of the common practice of preserving cultures of micro-organisms in media containing chalk, Komarova's (32) observations are of considerable interest. He found the rate of lactic acid fermentation to be considerably increased by the addition of chalk to the medium, explaining this by the occurrence of convection currents resulting from chemical reactions between the chalk and lactic acid, these keeping the bacteria in suspension, supplying them with nutrients, and removing their metabolites. The increase in lactic acid fermentation may be novel in

connexion with chalk as a media constituent, but the mode of action Komarova suggests could at least explain bacterial longevity and viability. In the preservation of bacteria by freeze-drying the composition of the suspending medium is generally one of the most important factors determining percentage survival (see Briggs, Tull, Newland & Briggs (33)), but Greaves (34) has now described an apparatus for drying at −30° C. in which the choice of vehicle is much less critical; he gives the formula for a medium containing dextran, peptone, glucose, and a detergent, 'Triton W.R. 1339', which gives very high percentage survivals. Annear (35) has continued his work on the preservation of bacteria by incorporating small volumes of suspension in plugs of sterile freeze-dried peptone; the method seems valuable for organisms, like *Neisseria*, that are particularly sensitive to drying. Various American authorities have contributed to a comprehensive general Symposium (36) on the maintenance of cultures of micro-organisms, and in the field of dairying Winkler & Jager (37) have shown conclusively the suitability of freeze-dried cultures for Emmental cheese manufacture. Similarly, Whitehead, Briggs, Garvie & Newland (38) compared two subcultures of a strain of *Str. cremoris* 'HP' after they had been maintained under different conditions in two countries for 18 years; the one had been freeze-dried, while the other was repurified frequently with deliberate selection of its more active components, but the two cultures were found to have retained their main characteristics unchanged; only small changes of degree were noted, including a slight loss in the acid-producing activity of the freeze-dried cultures. Valuable detailed studies of the factors influencing the survival of mixed starter cultures on freeze-drying have been made by Watts & Calbert (39); sterilized 10% reconstituted dried skim-milk was a satisfactory medium for both growth and drying, and cells in late logarithmic and early stationary phases of growth were at the best physiological age for drying. The activity of the dried cultures was prolonged by storage below 0° C., and good cottage cheese and buttermilk were made with direct use of freeze-dried cultures. It is of interest for those who need to re-dry cultures at fairly frequent intervals that, on repeated freezing and thawing of bacteria, the lethal effect is greatest during the second freezing period, whether this be 30 min., 24 hr., or a week or a month after the first (Harrison (40)); the nature of the suspending medium is particularly important in this connexion, Harrison's results using water as the vehicle being most erratic. Johns (41) has shown with frozen cheese starters that the survival rate and activity of cultures neutralized with NaOH before freezing were significantly greater than those of unneutralized controls, and Nikolic (42) has described the freeze-drying of various sour milk products, including yoghurt and kefir, and a proposed plant for their commercial production. Another technique for the preservation of micro-organisms involves mixing them with anhydrous Na_2SO_4, which absorbs water to form $Na_2SO_4.10H_2O$, the mass crystallizing and eventually forming a white friable or powdery mass (43). Yeasts so preserved retained their vitality for 4–5 years, and pure cultures were always obtained on reconstitution although no special precautions had been taken to ensure sterility during the crystallization process; data for moulds and bacteria have not yet appeared, and it may well be that contamination would present a greater problem with these than with yeasts. The maintenance of yeasts by freeze-drying, and the National Collection of Yeast Cultures have been described by Kirsop (44,45) and a Catalogue of strains maintained by the National Collection of Industrial Bacteria has been published (46). The first Catalogue of strains maintained by the newly established National Collection of Dairy Organisms has been published (47), the nucleus of the Collection being the various starter, spoilage and other organisms kept for many years in the Bacteriology Department of the N.I.R.D.

II. STARTERS

General

Compatibility of the component strains is of great importance in the selection of a mixed-strain lactic starter; on it will depend the stability of the starter. Thus Lightbody & Meanwell (1) found that two strains of *Streptococcus cremoris* or one strain of *Str. lactis* became dominant in each of the three-strain mixed starters selected from ten strains of *Str. lactis* and *Str. cremoris*. The strains

attained dominance by the secretion of a substance inhibitory to the growth of other strains. This substance was less potent than nisin and did not interfere with the cheese-making process. Dominance was sometimes reached within five subcultures, but twenty to sixty subcultures were needed for the complete disappearance of all other strains in the starter. On the other hand, East (2) noted that each of six relatively stable commercial starters consisted of three strains of varying capacities for acid-production and reduction. In pure culture all strains were slow acid-producers, but some strains produced water-soluble, heat-stable, growth-stimulating substances, and the acidity of the mixed starters was much higher than that of pure cultures of the constituent starters. Hall (3,4) has found that some strains of *Escherichia coli* secrete substances stimulatory to some strains of *Str. lactis* and she suggests that the casual infection of farmhouse starters with such strains of *E. coli* may have been a factor in the production of the cheese flavour associated with farmhouse cheese. *Str. lactis* soon became dominant in experimentally infected starters and *E. coli* soon died out in Cheddar cheese made with a coli-lactic starter. Hamdy, Harper & Weiser (5) have shown that the amino acid patterns of single-strain cultures in skim-milk of *Lactobacillus lactis*, *Str. thermophilus*, and *L. bulgaricus* are entirely different from those of the mixed-strain and mixed-species cultures of these organisms.

That general cultural conditions may affect the composition of the starter is suggested by the experience of Doležálek (6) who found that the flora of Danish butter cultures which, on arrival in Czechoslovakia, was *Str. lactis* 55 %, *Str. cremoris* 31 %, *Leuconostoc citrovorum* and *Leuc. paracitrovorum* 14 %, changed during cultivation in that country, the proportion of *Str. lactis* rising to 90 %, and *Leuc. citrovorum* and *Leuc. paracitrovorum* being no longer detected; acid-production decreased and the aroma was lost. The original properties were restored when the cultures were returned to Denmark and Danish management. The proportion of *Str. lactis* in Swiss cultures also increased under Czechoslovakian management, but Czech butter cultures remained stable. On the other hand, Whitehead, Briggs, Garvie & Newland (7) have recently compared two strains of the culture HP, one of which has been maintained for 18 years in New Zealand under New Zealand management which included periodical re-selection to maintain activity, the other under English laboratory control without the re-selection. The strains had proved remarkably stable and there were only slight differences between them. The English culture gave a slightly less active cheese starter, but no significant difference was detected between cheeses made with the respective starters. The use of nutritionally defective strains of lactic streptococci or nutritionally deficient milk may be a cause of 'slowness' in starters. Garvie & Mabbitt (8) were able to restore the activity of starters which had become slow on continued propagation in milk, by adding liver extract, yeast extract or peptone to the milk. The change was only temporary and the culture reverted to 'slowness' in the absence of the added stimulatory substance which appeared to be of the nature of a peptide or amino acid. It is probable that the organisms had lost a proteolytic enzyme. Anderson, Parker & Elliker (9) also found that acid production by single or mixed strain starters increased with an increased peptide content of milk. The effect varied with the strain. Greene & Jezeski (10) link the activity of starters with the amount of undenatured serum protein in the milk or reconstituted dried milk used as culture medium. Severe heat treatment in the preparation of dried milk was deleterious to its usefulness as a culture medium, and Olson, Beachboard & Cohenour (11) state that a high S.N.F. content of milk favours the production of active starters. Auclair & Portmann (12) have shown that the destruction of lactenin by heating milk at temperatures between 70 and 80° C. markedly improved it as a culture medium for strains of *Str. lactis*, *Str. agalactiae*, *Str. cremoris* and *L. lactis*. The growth of one strain of *Str. lactis* was not affected by the heat treatment of the milk.

If leuconostocs are components of starters they constitute only a small proportion of the flora, and since they tend to be overgrown by the lactic streptococci they are not readily detected by ordinary plating methods. They are of importance in Swedish butter and cheese starters, and Järvik (13) has evolved an enrichment method in which the starter is incubated in skim-milk fortified with autolysed yeast and buffered with acetic acid or α-bromopropionic acid. The leuconostocs tend to die out less rapidly than the streptococci and are therefore more readily

found in 7-day-old starters and 14-day-old cheese. Csongrády & Kiss(14) have devised an agar medium unfavourable for *Str. lactis* and *Str. cremoris* but giving colonies of *Leuc. citrovorum* and *Leuc. paracitrovorum* which are readily distinguishable and give characteristic physiological reactions.

Bacteriophage

Acid production in milk cultures has been widely used for the detection of phage in starters and whey. Valles(15) has found redox-potential indicators useful in the detection, purification and identification of phages, giving results comparable with plaque formation on agar media. Wilkowske, Nelson & Parmelee(16) used serological grouping in the identification of lactic streptococcal phages and found that the results agreed reasonably well with those obtained by the activity pattern method. They suggest a combination of the two methods. The heat resistance of these phages varied with the strain, but, within the limits of the small number of strains tested, was the same for all members of a serological group(17). Bacteriophages active against *Str. thermophilus* have been isolated from yoghurt cultures. The heat resistance of these phages and others isolated from starter cultures varied, but heat treatment which destroyed phosphatase in milk did not necessarily ensure its freedom from phage. Some survived in milk giving a Storch's reaction(18).

Strain dominance in starters due to the activity of phage has been studied by Collins(19,20). Acid production in a mixture of three strains of *Str. lactis* was reduced by phage activity only if two of the strains were attacked or where a sensitive strain had become dominant. The order of dominance of several strains could be established by exposing a mixture of the strains, after repeated subculture, to the action of the homologous phages added singly. Reduced acid production in the presence of a phage indicated the dominance of the homologous strain of *Str. lactis*. After this lysis of the dominant strain others rose to dominance in the order indicated by phage lysis. Commercial starters contained few unrelated strains and Collins(21) reports an instance in which phages active against all but one of a group of fresh starters were already present in the whey at a cottage cheese factory. This frequent incidence of lactic phages at soft-cheese factories has also been observed by Gibshman & Belousova(22). They were less frequent at hard-cheese factories and were found in only four out of sixty soil samples.

Czulak & Naylor(23,24,25) obtained a lysogenic strain of *Str. lactis* by lysing the parent strain with an homologous phage; this phage persisted through two subsequent strains obtained by lysing with two other phages neither of which was carried by these strains. The first two phages prevented acid production not only by the original strain of *Str. lactis*, but also by an unrelated strain of *Str. cremoris* growing with it—an action reminiscent of 'nascent phage'. No phage capable of lysing the strain of *Str. cremoris* could be isolated from the culture; the induced lysis of this strain was caused by lysin liberated from the phages during phage action. An indication of this was given by the formation of haloes round their plaques. It is suggested that similar lysogenic strains may arise under natural conditions, and the liberation of lysins from phages may throw new light on incompatibility between the components of a mixed-strain starter.

Difficulties due to phage activity occur most frequently in the preparation of starter. Galesloot(26) rarely experienced trouble in the early stages of cheese-making and found no difficulty, even when using a very sensitive starter, in making Edam cheese with milk heavily inoculated with phage particles (100,000–1,000,000 per ml.). He suggests that curd and fresh cheese protect the streptococci against the action of the phage. Czulak(27) had observed, some years previously, that if phage was added to the cheese curd it had no effect on the development of the cheese, although the phage was not destroyed and could be isolated from the maturing cheese. These findings are supported by the experiments of Meanwell & Thompson(28) who found that the addition of rennet to milk cultures, within 30 min. of the addition of phage, delayed phage action by several hours. The degree of protection afforded by the rennet depended on the rate of multiplication of the phage. The growth of streptococci was stimulated and phage multiplication retarded, thus prolonging the interval before the numbers of cells and phage particles were equal,

i.e. when phage activity normally begins. The protection offered by the rennet clot was equal to that of milk coagulated with agar, supporting the view that the protection was determined by the physical properties of the medium.

In earlier reviews reference has been made to the fact that Ca is essential for the proliferation of bacteriophage and to the use of Ca-free whey or synthetic media for starter propagation. Reiter (29) has introduced a Ca-free medium with a milk base for this purpose. A high proportion of commercial and single-strain starters grew normally in it but phage did not multiply. Normal Cheddar and Cheshire cheese were made with starters propagated in this medium even in phage-infected dairies.

Antibiotics

Reports of the isolation of nisin-producing strains of lactic streptococci and their use as cheese starters in Poland and Hungary emphasize the wide-spread distribution of these strains. Four strains of streptococci belonging to serological group N, isolated from cows' and ewes' milk by Lipińska, (30), produced an antibiotic similar to nisin and inhibitory to butyric acid bacteria. The addition of one of the strains to cheese milk, artificially infected with butyric acid bacteria, prevented butyric acid fermentation in Edam cheese manufactured from it. Csiszár & Pulay (31) found that the action of six nisin-producing strains of *Str. lactis* was bacteriostatic and that the clostridia resumed growth on prolonged incubation of the cultures. When these strains were used as starters in the manufacture of cheese such as Ovar (a Tilsit-like cheese), Port du Salut, Pannomia or Emmental from milk heavily infected with spores of *Clostridium tyrobutyricum*, the rate of acid-production was reduced and the consequent slower drainage, linked with the slower removal of lactose, increased the danger of early blowing by *E. coli* (32) in quick-ripening cheese. Moreover, Pulay (32) reports that glucose could be detected in the Emmental cheese. In the long-ripening types there was danger of 'late blowing' due to the progressive destruction of nisin and eventual renewed growth of the clostridia. While making Edam and Gouda cheese with raw milk and a nisin-producing starter, Galesloot (33) noted a destruction of nisin within 48 hr. which could not therefore be due to *L. plantarum* as previously reported by Kooy (34). About 50% of the lactic streptococci (mostly *Str. lactis* or *Str. cremoris*) isolated from raw milk destroyed nisin in a nisin-producing starter. It would appear that production of nisinase is a wide-spread characteristic of lactic streptococci, but it can function in mixed starters only in those strains in which it is produced rapidly enough to destroy the nisin before the critical pH for nisinase activity is reached.

Two methods are proposed for the detection of nisin in starters and cheese: (i) The reduction of litmus in milk to which a sterilized filtrate of the starter culture or cheese emulsion has been added together with the test organism—Galesloot & Pette (35). (ii) The development of a clear zone around a cylinder of cheese placed in a Petri dish and covered with agar inoculated with a sensitive organism—Mocquot & Lefebvre (36).

With the increasing use of nisin in cheese manufacture there is a wide demand for reliable supplies of a dried stabilized culture of a nisin-producing strain of *Str. lactis*. Hawley (37) discusses some of the problems encountered in the production of such a culture on a manufacturing scale. Difficulties may still arise in its use. For instance, while Edam cheese could be successfully made with the use of a nisin-producing starter the inhibition of the lactobacilli disturbed the normal ripening of Gruyère. Some strains of *L. casei* will, without actually destroying nisin, nullify its action on the clostridia. The protective action of nisin in processed cheese may thus be invalidated if such strains enter as post-processing contaminants.

In the detection of *penicillin* in milk and milk products Galesloot (38) used *Str. thermophilus* as the test organism and methylene-blue reduction as the indicator. Matthews & Hesketh (39) used *Sarcina lutea* streaked on agar slopes containing the milk under test. Cerny & Morris (40) used impregnated filter-paper disks lightly pressed on agar plates seeded with spores of *Bacillus subtilis*. Whitehead & Cox (41) used the change in the morphology of *Str. cremoris* grown in the presence of penicillin. Unmistakable bacillary forms were induced by 0·1 i.u./ml. of penicillin,

the slightly swollen forms induced by 0·05 i.u./ml. were less clearly defined. *Str. thermophilus* would detect 0·01 i.u./ml., but such a low concentration of penicillin is of little significance for cheese-making. According to Whitehead & Lane (42) the presence of 0·05 i.u./ml. of penicillin in the cheese-milk prolonged manufacture by about 25 min. but did not appreciably affect the quality of the cheese. A concentration of 0·1 i.u./ml. appeared to be critical; some of the cheese were normal, others of inferior quality. Cheeses made with milk containing 0·15 i.u./ml. were all of poor quality. Galesloot (43), too, observed that strains of *Str. cremoris* and *Str. lactis* appeared as chains of rods when grown in the presence of 0·3–0·5 i.u./ml. In yoghurt cultures *Str. thermophilus* was often entirely suppressed and *L. bulgaricus* grew as long filaments of drawn-out rods. A microscopic examination of abnormal yoghurt would indicate whether the fault was due to penicillin or to phage which causes other quite distinct morphological changes. Adamse's (44) data agree with others in that 0·05 i.u./ml. of penicillin in milk disturbed, and 0·3 i.u./ml. almost entirely inhibited, acid production in starters. Yoghurt cultures were affected by as little as 0·005 i.u./ml. Penicillin in milk was not destroyed by pasteurization. Shahani, Gould & Weiser (45) found that loss of potency on heating varied with the type of penicillin and was greater in water or phosphate buffer than in milk. There was further loss of activity on storage at 34–38° F.

In view of the unsuitability, for cheese-making, of antibiotic-containing milk, surveys of the incidence of penicillin and other antibiotics in milk supplies have been made for several districts. Overby (46) has reviewed early surveys and Berridge (47,48) has co-ordinated the results of a joint survey of eight districts covering a wide area of England and Wales and comprising 5000 samples taken from vat, churn or farm. Penicillin was present in concentrations of 0·04 i.u./ml. or more in 3·7% of the farm samples and in 2·3% of the samples of bulk milk from tankers, storage tanks and cheese vats. The sensitivity to penicillin varied widely for individual strains of *Str. lactis* and *Str. cremoris* (49). Acid production was not affected in six strains until penicillin was present in a concentration of >0·096 i.u./ml., while three strains were slightly inhibited even by a concentration of 0·012 i.u./ml. The quality of experimental Cheshire cheese was not affected by concentrations of penicillin below those that affected the activity of the starter. Panes, Edge, Hobson & Thomas (50) examined samples of cheese-milk from four Cheshire cheese creameries in Wales, also of bulk milk from weigh-tanks and of milk used for starter propagation. Penicillin in concentrations of 0·05 i.u./ml. or more was found in 3·1% of the farm milk supplies, 3·4% of the vat samples and 4·2% of the samples set aside for starter production. Neither these authors nor Berridge found an obvious seasonal trend in the incidence. Earlier experiments by Thomas, Panes & Lewis (51,52) indicate that the 'critical level' of penicillin for Cheshire cheese manufacture lies between 0·05 and 0·15 i.u./ml. Whitehead & Lane (42) suggest that the 'critical level' for Cheddar cheese is 0·1 i.u./ml. Meewes & Milošević have also reported that a concentration of 0·1 i.u./ml. of penicillin in the cheese-milk adversely affected the quality of Tilsit (53) and Romadur (54) cheese. Normal Cheshire cheese could be made from the milk of cows receiving daily infusions of penicillin if the milk was treated with penicillinase—Chapman (55). Whitehead & Lane (42) question the economic soundness of penicillinase treatment under commercial conditions. Although 7·3% of samples of raw milk in the Columbus, Ohio, area (56) contained antibiotics of various kinds, the concentration was critical in only 1·3%. In Wisconsin (57) 4·3% of samples of herd milk as received at thirty dairy plants caused a 20% reduction in acid-production by *Str. thermophilus*. In a second survey 2·4% of the samples were strongly, 6·3% mildly and 17·8% slightly inhibitory.

The presence of leuconostocs in mixed starter cultures is desirable when they are to be used for the manufacture of ripened-cream butter or certain types of cheese. Leuconostocs are, however, less sensitive to penicillin than the lactic streptococci and would, therefore, become dominant in starters and cheese made with penicillin-containing milk, with resultant active gas production—Järvik (58). In cultures in normal milk the lactic streptococci remain dominant, but they produce substances stimulatory to the growth of the leuconostocs, which slowly increase in numbers and at 24 hr. constitute a much higher proportion of the flora than at the outset,

especially if citric or lactic acid has been added to the milk—Glenn & Prouty (59). This stimulation of growth of leuconostocs by lactic streptococci has also been the subject of experiments by Deryabina (60). Holwerda (61) noted an upward trend in the growth curves of leuconostocs in starters when the number of streptococci reached a certain level, but the growth of both species ceased when pH 4·8 was reached. In strongly reducing starters the early rapid growth of leuconostocs and fermentation of citrate while the acidity was still low resulted in too great a reduction of acetoin and too little residual diacetyl in the ripe starter. The late growth of leuconostocs in weak starters gave conditions more favourable for the development of a starter with a diacetyl content suitable for butter-making.

III. CHEESE

Cheddar cheese manufacture

Further trials have been made of the Australian short-time method for Cheddar cheese manufacture. Czulak & Hammond (1,2,3) report that it has been used on a commercial scale in six dairies in Australia and that during 1954–55 2500 tons of cheese were made of a quality comparable with that of cheese made by the old conventional method. A strain of *Streptococcus durans* exceedingly active at 45° C. was comparable with *Str. lactis* at 30° C. and *Str. thermophilus* at 45° C. When used in conjunction with *Str. cremoris* it contributed to acid development in the vat and press. It was used in preference to *Str. thermophilus* because of the greater sensitivity of the latter to NaCl. A strain of *Str. thermophilus* has, however, now been isolated which has almost twice the acid-producing capacity of *Str. durans* and this has been tried in cheese-making. An acidity of pH 5·1–5·3 as the cheese leaves the press is desirable. This should be attained with a starter consisting of 2% normal + 2% fast thermoduric culture, but it has been found possible to correct slow acid development by extending the period of the first cooking at 100° C. Feagan (4) omitted the normal starter and used only 2·0–2·5% active strains of *Str. thermophilus*. The first cooking temperature was raised to 106° F. in 40–45 min. Downs (5) in the U.S.A. reports the manufacture of cheese, on a commercial scale, by the Australian short-time method. Those made with pasteurized milk were of a quality comparable with those made by the conventional method, but those from raw milk were of open texture with Swiss eye-formation. Crawford (6), in small-scale trials in Scotland, added *Str. durans* or *L. bulgaricus* to the normal starter. The cheese always matured more slowly than the normal, but were of good body and texture. The use of *L. bulgaricus* instead of *Str. durans* as starter gave cheese of better and stronger flavour. Sharkey & Patten (7) describe how, by mechanization and good management of the process, good quality Cheddar cheese have been made from 50,000 gal. of milk daily in a factory where only seventeen vats were available; the average production over a period of 3 months was forty-three vats daily. The mixture of curd and whey was pumped from the vat, the whey drained off and the curd transferred direct to the cheddaring vat. Cheddar cheese made from milk to which 0·25% H_2O_2 had been added 24–30 hr. before cheese manufacture and removed by catalase before pasteurization were unsatisfactory. Some improvement was found when H_2O_2 was added in small amounts at intervals, and cheese of better quality still were obtained when a mixture of treated and untreated milk was used—Lück (8).

Cheddar cheese ripening

The importance of the streptococci lies chiefly in connexion with starter preparation and acid development in the early stages of manufacture. Current literature has been reviewed in the section dealing with starters.

The lactobacilli are the prime agents in the ripening of cheese—at least in the hard, pressed varieties. Mabbitt and his colleagues have contributed to the understanding of their role. Mabbitt & Zielinska (9) devised a medium, a modification of Rogosa's, in which a tryptic digest of milk is substituted for tryptic digest of casein, and the general ingredients are increased by 10%. All lactobacilli normally found in cheese grew well in this medium, but the streptococci

were inhibited. With the use of this medium the numbers of lactobacilli could be estimated and their growth studied in the earliest stages of cheese-ripening. In comparing this medium with that of Rogosa and tomato dextrose agar, Naylor(10) found that most species of lactobacilli which grow at 30° C. grew better in the acetate broth than in tomato dextrose broth, but for those growing at 37° C. tomato dextrose broth was better.

Wheater(11) has differentiated strains of *L. plantarum*, *L. casei* and *L. helveticus* on the basis of physiological tests. Sharpe(12) has used serological and physiological criteria for the identification of lactobacilli isolated from various types of cheese, while Mattick, Cheeseman, Berridge & Bottazzi(13) have used the amino acid and peptide patterns (as shown by paper partition chromatography) of acetic acid extracts of the cells. The patterns were relatively constant for closely related strains within a species, but differed for species. There are indications that this technique may offer a means of distinguishing between *L. brevis* and *L. fermenti* and between *Str. lactis* and *Str. cremoris*, species which are normally difficult to differentiate.

Mabbitt & Zielinska(14) found that the streptococci in a ripening Cheddar cheese were at a maximum in the early stages, but fell to a minimum at 4–5 months, i.e. at a time when the lactobacilli had gained predominance. The serum expressed from mature (6–15 month) cheese would not support the growth of a test organism, *L. brevis*, unless the serum was autoclaved or diluted with water; the addition of glucose, peptone, casein digest or catalase did not induce growth. The only sera in which the organism would grow were those expressed from a 4–6-month-old cheese, i.e. during a period which approximately coincided with that of active growth of the lactobacilli in the cheese. Mabbitt & Zielinska suggest that at this period the proportion of amino acids in the serum was favourable for the growth of the lactobacilli; before that time it was unfavourable, after that time the increasing osmotic pressure of the serum inhibited the growth of these organisms. Mabbitt(15) has estimated the free amino acids in Cheddar cheese by means of column chromatography. In ripe cheese they reached a value of 75–100 mg. per ml. of serum. The concentration of basic amino acids was relatively high, that of proline low. A mixture of amino acids comparable with that found in ripe cheese gave a 'brothy' flavour to fresh curd, and a similar flavour developed in cheese made from skim-milk(16). The typical flavour of Cheddar cheese appears to be concentrated in the fat, but the actual flavour substances and the effect of differences in the bacterial flora have still to be clarified. Kristoffersen & Nelson(17,18) isolated from American Cheddar cheese strains of *L. casei* capable of deaminating serine and of producing H_2S. Not all strains showed deaminase activity, but at least one H_2S-producing strain was isolated from each of the cheese with the highest flavour scores. Other experiments had shown that twice as much NH_3 was liberated from L-serine as from DL-serine, and phosphoserine was not deaminated at all. The authors suggest that deamination of amino acids may offer a source of energy for lactobacilli after the sugars have been exhausted, and the fact that phosphoserine is not deaminated may explain the lack of flavour in pasteurized milk cheese. In experimental cheese made from pasteurized milk to which serine or phosphatase had been added, the highest flavour scores were awarded to those with the highest H_2S content at 6 months. Those to which serine or phosphatase had been added were superior to others lacking these additions.

Yates, Irvine & Cunningham(19) found that a number of the bacteria isolated from milk, cheese and whey were proteolytic; the activity was increased if rennet was added to the milk. Flavour improved and the ripening was accelerated when *L. casei* was added with the starter, but a bitter flavour and poor texture developed when *Str. liquefaciens* was added. The rate of growth and proteolytic activity of *L. casei* has been studied by Brandsaeter & Nelson(20,21). The optima for growth and proteinase activity in milk were at pH 5·5–6·5. Although the maximum activity of the cell-free extract was at pH 5·5–6·0, 50% of this remained at pH 5·0. The optimum for peptidases was at pH 7·0–8·0 and the maximum stimulation of the peptidases by Co^{++} was at pH 5·5. The normal pH range in Cheddar cheese is thus favourable for these enzyme actions.

'Red spot' periodically recurs in Cheddar cheese otherwise of good quality. No current account of direct researches on the problem has been seen, but the investigation of Swedish

scientists showing the danger of slow fermentation and residual sugars in the fresh cheese, and control measures suggested for Herrgård and Svecia cheese may be of interest to Cheddar cheese-makers.

Silverman & Kosikowski (22) have described a technique for the estimation of tyrosine in cheese by decarboxylation to tyramine with dried cells of a strain of *Str. faecalis*. Using this method they demonstrated (23) that the amount of tyrosine liberated in Cheddar cheese steadily increased with the age of the cheese. The rate of its conversion to tyramine varied and was greatest in cheese in which there was an active decarboxylase system. Their results support the view that tyrosine would be better than soluble protein as an index of the degree of cheese-ripening.

Other cheese

Edam and Gouda

Reference has already been made above to the fact that H_2S-producing strains of *L. casei* were isolated from Cheddar cheese with the highest flavour scores. Pette (24) also has remarked that H_2S may contribute to the typical flavour of Gouda cheese. In some, especially farmhouse cheese, however, the amount of H_2S was excessive. It was produced by streptococci which closely resembled *Str. faecalis* although not identical with it. Pette has proposed the name *Str. faecalis* var. *malodoratus*. De Man (25) has studied strains of lactobacilli isolated from Dutch cheese, and has classified them on the basis of Orla-Jensen's scheme. The homofermentative strains formed two species, *Streptobacterium* (*lactobacillus*) *casei* and *Sbm. plantarum*. The relative frequency of these strains in cheese was not determined, but it was noted that *Sbm. casei* was not isolated from cheese containing nisin. Some of the heterofermentative strains fermented citrate, and it is suggested that in the absence of leuconostocs these may contribute to the eye-formation in Dutch cheese. Small round pustules in the coats of Dutch cheese (26) were due to the proteolytic activity of a mould, *Geotrichum candidum*, growing in combination with yeasts and bacteria.

Herrgård and Svecia

Two frequent faults in these Swedish cheese are 'early blowing' and 'red spot'. Their causal organisms are different, but the basic cause of each is the incomplete removal of sugars in the early stages of manufacture, and this, in turn, is linked with the scalding temperature. Sjöström & Guldstrand (27) observed by means of paper chromatography that when a scald temperature of 44° C. was used the lactose rapidly disappeared but the galactose persisted, often for 22 days. With a scald temperature of 39° C. both lactose and galactose disappeared within 2–3 days. They suggest that the heat treatment with the higher scald temperature impairs the ability of the lactobacilli to ferment galactose. Swartling & Lindgren (28) found that some of the bacteria in a starter actively growing at 30° C. were destroyed if it were transferred to 40 or 45° C. and that the numbers of lactic streptococci in experimental cheese scalded at 40° C. were higher than in the control cheese scalded at 45° C. The fermentation of the residual sugar by coliform organisms is the cause of 'early blowing'. The production of the red pigment by *L. plantarum* var. *rudensis* is also linked with the presence of sugar. The defect 'red-spot' develops if colonies large enough to be visible to the naked eye develop before the sugar is exhausted—Thomé (29). Swartling, Lodin & Nilsson (30) controlled the fault in a Svecia cheese factory by reducing the scald temperature to 40° C. Control cheese using the normal factory temperature, 44° C., were seriously discoloured. Swartling & Lindgren (28) give a warning that this procedure may alter the characteristics of the cheese, and suggest that the effect of heavier starter inoculum and selection of more active strains of greater heat-resisting capacity should also be examined.

'Surface pitting' in Svecia cheese was traced to the growth of strongly proteolytic moulds such as *Penicillium* and *Mucor*. Large numbers of lactobacilli usually were found in the vicinity of the moulds. Brine salting and early waxing controlled the fault, but this is not normal procedure for Svecia cheese (31).

Emmental

The manufacture of Emmental cheese was introduced into Finland from Switzerland in 1856. The centenary has been celebrated by exhibition of original equipment and reviews of developments during the intervening 100 years (32). Antila (33) has isolated propionic acid bacteria from Emmental cheese and identified eleven species; strains of these species showed differences in properties of value in cheese-making. The use of a starter containing *Propionibacterium shermanii* for cheese made with raw milk did not affect the propionic acid bacterial flora; wild strains were found even when the cheese milk was pasteurized. The citrate-fermenting capacity varied with the strain.

'Red spot' in Emmental cheese is caused by large colonies of red or orange pigment-forming strains of propionic acid bacteria. In a study of this problem Antila & Hietaranta (34) have shown that numbers of bacteria are more important than strain. The defective cheese contained slightly more propionic acid than the normal, otherwise the chemical composition was little affected.

Variations in the amount of amino acids were found for Swiss cheese made from raw milk using a starter containing *Str. thermophilus*, *L. bulgaricus* and *Propionibacterium shermanii*, but proline and propionic acid appeared to be closely linked with the typical cheese flavour; butyric acid was linked with poor flavour. The propionic acid fermentation occurred in the early stages of ripening (35). Willart (36) found that a greater amount of lipolysis occurred on the coats than in the interior.

Mould-ripened cheese

The majority of the lactobacilli isolated from the coats of Stilton cheese were identified as *L. plantarum*. *L. brevis* and *L. casei* were found in relatively small numbers—Sharpe & Brindley (37). The samples were from four dairies, from cheese made with raw and pasteurized milk and even from a slip-coat cheese. Yet twenty-four of the thirty-two strains of *L. plantarum* were of a single serological type and were predominant on the coats of the cheese from each dairy.

A characteristic feature of the blue-veined cheese is the liberation of fatty-acids by lipases of *Penicillium roqueforti*. Morris, Jezeski, Combs & Kuramoto (38) found that the time at which the characteristic flavour of blue cheese developed and the proportion of butyric acid to caproic + higher acids varied according to whether the cheese-milk had been raw, homogenized, or pasteurized-homogenized. The ripening period was longest and the quantity of caproic + higher acids lowest for the raw milk cheese. The component fatty acids of the liquid and solid fractions of butter oil are similar, but the lipase activity of *P. roqueforti* was greater in the liquid than in the solid fraction (39). Wilcox, Nelson & Wood's (40) experiments with various micro-organisms indicate the specificity of their lipases. *Candida lipolytica*, for instance, liberated butyric and caproic acids from butter oil (one strain liberated butyric only). *P. roqueforti* liberated butyric, caproic and caprylic acids. Methyl ketones were formed by oxidation of the fatty acids by the moulds. Morgan & Anderson (41) identified methyl-propyl-ketone, methyl-amyl-ketone and methyl-heptyl-ketone in varying amounts in French Roquefort, Danish Blue, Domestic Blue and Gorgonzola cheeses of typical flavour and aroma. Methyl ketones were produced from the sodium salts of fatty acids by resting cells of a culture of *P. roqueforti* used in cheese-making (42). The methyl-ketones are themselves inhibitory to the growth of the mould, and it is possible that the methyl-ketones produced in the blue-veined cheese are a factor in preventing excessive mould growth.

Violet spots underneath the mould mat of cheese such as Camembert were found by Keilling, Casalis, Savignac & Dubreuil (43) to be due to the growth of *P. funiculosum*. Fat appeared to be essential to the development of the violet colour. The most extensive discoloration occurred when there was early infection of the cheese surface and the *P. funiculosum* was well established before the inoculation with *P. candidum*.

Cottage cheese

There is serious danger of post-pasteurization contamination of cottage cheese. The keeping quality is related to the numbers of coliform and lipolytic organisms. The pH is a controlling factor in the survival of these and other undesirable bacteria (44,45). *Pseudomonas fragi* is a frequent cause of spoilage of commercial cottage cheese (46).

Surface treatment

The microflora of the surface smear of Brick cheese has been investigated by Lubert & Frazier (47). *Micrococcus varians* (constituting 80% of the cultures), *M. caseolyticus*, *M. freudenreichii* and four types of film yeast induced the development of the typical smear. It was essential that the yeasts should develop first to reduce the pH to a level suitable for the growth of the micrococci and to provide nutrients and growth substances. The characteristic flavour and odour of the smear were due to fatty acids produced by a mixture of the micrococci and yeasts.

It has been the custom in Denmark and the Netherlands to treat cheese surfaces with mineral oil to develop a typical coat and prevent surface mould growth. The use of mineral oils has now been forbidden, but vegetable oils have proved unsatisfactory substitutes for this purpose. It is now recommended that, to develop a typical smear on cheese such as Havarti (Danish Tilsit), the surface shall be treated with a smear culture, an emulsion of a typical cheese, or brine and whey. The moisture content of the cheese and the humidity of the ripening room would then be of great importance (48, 49, 50, 51, 52).

Various foil and plastic wrappings for Camembert cheese have been tested (53); a plastic layer applied to the wax coating of hard cheese reduced moisture, but did not prevent mould growth unless it was thickly applied (54). Hendrickx & de Vleeschauwer (55) observed that there was little advantage in using citrate solution instead of water in preparing samples of Saint-Paulin, Gouda, Camembert and processed cheese for bacteriological analysis.

Another volume of the *Manuale Lactis* has been published (56). It contains references to the literature on cheese that has appeared during the years 1948–55, classified according to the *Bibliotheca Lactis* system. The small book on *Cheese and Butter*, by Cheke & Sheppard (57), gives an insight into the manufacture of cheese at the factory, on the farm and in the home, with recipes for some varieties.

IV. BUTTER

Two microbiological techniques, the Frost Little Plate and microtome sectioning, have been adapted to the examination of butter. In the former, used by Gavél & Demeter (1,2,3), a drop of a mixture of the butter serum and agar forms the plate. An insight into the flora of the butter may be gained by varying the formula of the agar medium and the time and temperature of incubation, and from microscopic examination of the young colonies. The second method, microtome sections, as described by Gavél (4,5), primarily illustrates the structure of the butter, but the microorganisms are readily examined in stained sections. In this way Gavél has observed that the intensity of the growth of the microorganisms was closely linked with the butter structure. These methods are also described by Demeter in his book *Mikrobiologie der Butter* (6), which covers a wide field, discussing the microflora of butter in general, its effect on the quality of butter and the factors which determine its numbers in butter.

Some countries have recently experimented with the addition of yeasts to butter with a view to improving its quality. Thus Bogdanov (7) and Blok & Geĭmberg (8), in Russia, found that experimental butter, to which asporogenous yeasts isolated from butter of long-keeping quality were added with the butter culture, was of longer keeping quality than the controls. Mašek, Maxa & Vedlich (9) in Czechoslovakia, added *Cryptococcus* spp. with the butter culture; these yeasts produced substances which delayed chemical oxidation of the fat and prevented the growth of aerobic moulds. The same authors (10) report that the incorporation of yeast cultures into the

butter after working to dryness also increased the keeping quality. Sándor(11) has suggested that, during prolonged working of butter to give a fine dispersal of moisture, bacteria adhere to and are eventually incorporated in the fat surrounding the serum droplets, thus reducing the danger of bacterial deterioration.

V. MILK CONTROL

'The problem of ensuring an adequate and wholesome supply of milk is a matter which concerns an ever-increasing number of countries...'. So begins the Foreword to *The Principles of Milk Legislation and Control*, by W. A. Lethem(1), Consultant to F.A.O., which should go far to help solution of that problem. There are many books about milk, but few about its control, and it is therefore particularly fortunate that this one should be succinct and informative, assuming the intelligence, but not the erudition of the reader, and easily amenable to translation; in fact, an excellent comprehensive publication, including a chapter on 'special provisions for hot climates', of world-wide value. The fourth edition of Chalmers's well-known book *Bacteria in Relation to the Milk Supply*(2), appearing 10 years after the third, has been revised and enlarged considerably in the light of recent investigations into the bacteriology of milk, and the 1939 edition of *Bacteriological Examination of Water Supplies*(3) has been carefully revised and partly re-written, now running to 54 pages. Mocquot's review(4) of 'technical and hygienic aspects concerning liquid milk' covers pasteurization, sterilization and keeping quality; the author advocates wider use of pasteurization with stricter control measures. Tentoni(5) has reviewed the various tests for evaluating the chemical and bacteriological quality of milk, and Mossel & Ingram(6) the physiology of the microbial spoilage of foods, including milk and milk products.

The removal of bacteria from milk by centrifugation has received much attention in the last 2 years, following the work of Simonart & Debeer(7), now reported in full at the XIVth International Dairy Congress(8). Although Gebhardt(9) has calculated that it is impracticable, for reasons of safety, to run conventional commercial separators at speeds necessary to eliminate a reasonable proportion of bacteria from milk, Lembke & Gantz(10) consider that such a procedure may become, in fact, commercially practicable. Much detailed work has been done by Arph(11), relating different combinations of time and speed to the degree of removal of organisms, particularly spores, from milk.

It is not proposed to discuss in detail here standards and legislation for milk, since a considerable part of the proceedings of the XIVth International Dairy Congress (1956) was devoted to this topic, numerous papers on various aspects of the subject being published in Volume III of the Proceedings of the Congress; one of the reports is by Lethem(12), on 'the principles of milk legislation for safety, quality and freshness'; a summary of British Standards concerned with dairy technology published since 1951 has appeared in the *Journal of the Society of Dairy Technology*(13). In France, owing to a change in the regulations, it is now only necessary to count the indole-producing coliforms in milk, which Thieulin & Basille(14) consider does not give a satisfactory assessment of its cleanliness; this may well be true in view of the frequency with which *Bacterium aerogenes* and other indole-negative varieties occur in the gut and faeces(15). Payment for milk on the basis of bacteriological quality is still a vexed question, but a detailed scheme has been proposed by de Vleeschauwer and his colleagues(16) in Belgium, who recommend a marked price differential between three quality grades, and Sainclivier(17) has developed a correction curve for the effects of atmospheric temperature for use in this connexion.

The now considerable literature on thermoduric organisms in milk has been reviewed by Thomas & Thomas(18), who studied 205 strains of microbacteria from milk-can rinses, finding 197 strains (96·1 %) of *Microbacterium lacticum*, 7 of *Mbm. liquefaciens*, and one of *Mbm. flavum*. The source of such organisms may be reflected in the work of Alford, Wiese & Gunter(19), who examined more than 2000 cultures of diphtheroid organisms, many heat-resistant, isolated from the reproductive tracts of dairy animals, and questioned the validity of the separation of the genera *Microbacterium* and *Corynebacterium*. Bhat & Bilimoria(20) have continued their work on the interactive effect on thermophiles of growth temperature and nutritional environment.

The micrococci in Indian milk supplies have been identified and characterized in some detail (21), *Micrococcus caseolyticus* being by far the most dangerous in terms of milk spoilage; micrococci represented about 72% of the total flora of fresh milk, and up to 60% of the thermoduric flora although only 9–20% of the total micrococci were heat-resistant (22). Little work on applied aspects of psychrophilic bacteria has appeared since Jezeski (23) in 1954 and Ljunggren (24) in 1956 reviewed the literature on these organisms, though Prouty (25) has studied the incidence of facultative psychrophiles in milk produced under the farm bulk tank system, and van der Zant & Moore (26) have made observations of practical importance with strains of *Pseudomonas*; proteolysis was always more rapid at higher temperatures (25–35° C.) and, although no direct relationship between numbers of organisms and proteolytic activity could be established, proteolysis was marked when plate counts ranged from 10^5 to 10^9 per ml.

Direct tests

Galesloot (27) and Overby (28) have reviewed the various tests, and their suitability for different purposes, used for grading milk according to hygienic and manufacturing value, and detailed proposals for standard tests for the examination of milk and dairy products in Germany have been set out in a report from the microbiological section of the German Methods Commission (29). The effects of the addition of seven different quaternary ammonium compounds (Q.A.C.) to raw milk have been studied by Clegg & Sarjeant (30), with a variety of keeping-quality tests; they showed that it is possible for low-quality milk to pass the 10 min. resazurin test with the aid of added Q.A.C., though there is a risk of premature coagulation.

'Bacto-strips'—strips of absorbent paper impregnated with a suitable bacteriological nutrient medium—afford a quick and simple method of demonstrating the presence of micro-organisms in milk, and are particularly convenient in routine screening tests (31); 8–10 hr. incubation times for exposed strips give the best results, since thereafter the colonies on the paper tend to disperse (32). With appropriate modifications of media and technique the method should enjoy a wide application, and is already of proven value for the detection of coliform organisms (33, 34, 35). The importance in dairying of identifying *Bact. coli* has been discussed by Lorenz (36), and it is probably true to say, again, that not only indole-positive coliforms are of significance (see Thieulin & Basille (14)); Thomas (37) has reviewed very fully recent work on the source, incidence and significance of organisms of the coli-aerogenes group in milk. A most interesting radioisotope technique for the rapid detection of coliforms has been developed by Levin, Harrison, Hess & Gurney (38) based on the fermentation of radioactive lactose with the production of $C^{14}O_2$; this is treated to precipitate in the form of barium carbonate which is then counted for radioactivity in a gas-flow Geiger counter. In the present state of development of the test, positive results with very small numbers of organisms can often be obtained within 10 min.; further progress with so rapid a qualitative test will be watched with considerable interest. Klein (39) has shown that for the estimation of coliform organisms in milk TTC agar (triphenyl tetrazolium chloride) gives high counts, but that many of the bacteria growing on this medium, although Gram-negative, are not in fact coliform organisms (40). An excellent empirical medium for coliforms has been devised by Bratlie & Fodstad (41), who observed that several commercial liquid detergents were heavily contaminated with colibacteria, and assumed that the detergents contained a selective substance; this proved to be alkyl-aryl-sulphonate, various of which compounds differed only slightly in their selective properties, and whose incorporation (1·2%) in a basal medium gave a better selective effect for coliform organisms than many selective coliform media previously used. Concerning streptococci as indicators of faecal contamination (see Hiscox & Briggs (42), p. 405) Cooper & Ramadan (43) have shown with physiological tests of 195 strains from human, bovine and sheep faeces that it is possible to trace the source of infection of water, milk and other foods; this may be better achieved by serological means, but such facilities are not, of course, so readily available. Little new work has appeared on the application of the membrane filter to the estimation of coliform organisms in milk, perhaps because of the persistent

difficulty that biological fluids tend to clog the pores of the filter; the apparatus is useful, however, in the bacteriological examination of water supplies (44), which Cuthbert (45,46) and Cuthbert, Scarlett & Westwater (47) have reviewed, though unsatisfactory for the estimation of *Bact. coli* in waters containing appreciable amounts of copper or zinc salts (48).

Indirect tests

The literature on dye-reduction tests for the control of the bacteriological quality of milk has been considerably less during the 2 years under review than in recent corresponding periods; Tentoni & Donnini (49) have written a very detailed and comprehensive review of the subject. Charlett (50) has discussed the methylene-blue test and, by the addition of 0·25 ml. of 'Teepol' to the tubes before adding the milk and dye, has eliminated the necessity for regular 30 min. inversion of the tubes (51), the fat remaining evenly distributed throughout the samples. Bleier & Brandner (58) have produced useful 'conversion' graphs relating the resazurin and methylene-blue reduction times of average milks, and Damude (53) has described a modified 1 hr. resazurin test, designed to suit field conditions, that the farmer can use himself with the appropriate test kit. The effect of environmental temperature on milk rejection tests, in particular dye reduction tests (54), has been studied in some detail by Sainclivier (55), in developing a method of effecting the appropriate correction; from his results with alcohol tests he had devised a scheme whereby the alcohol test score, obtained for milk at the time it reaches the dairy, may be adjusted according to the prevailing mean atmospheric temperature to indicate the bacteriological quality of the milk at the time of production. Jones & Thomas (56) have also examined alcohol tests, with a number of others, concluding that a satisfactory combination for the rapid sorting of raw milk is a paper strip pH indicator with an alcohol or rapid acidity test, e.g. using phenol-phthalein. Since 1 October 1955, Norwegian dairies have been allowed to substitute tasting for dye reduction tests, twelve times per year, deductions from quality payments being made for milk with faults of taste and smell as for milk low-graded by reductase tests (57); how far this concession will be adopted, and its effect on milk quality, remain to be seen. Thomé, Samuelsson & Mattsson (58) consider the methylene-blue reduction test quite unsuitable as a basis for paying bonuses for high-quality milk, though useful as a screening test for rejection of milks with reduction times of more than 2 hr. Even in this connexion, however, it seems that the results of tetrazolium reduction tests show better agreement with bacterial counts than do those of the methylene-blue test (59) and, moreover, with low-count milks tetrazolium gives a reaction more rapid than that of methylene blue, and can be used at room temperature (60); a greater advantage still is that, although sensitive to light, the formazans of tetrazolium salts are stable against oxygen, whereas methylene blue is readily re-oxidized by atmospheric oxygen.

VI. DETERGENTS AND DISINFECTANTS

Recommendations for the use of Detergents in the Dairying Industry (1), British Standard 2756, was published in 1956, and is primarily concerned with the cleaning of dairy equipment used for handling liquid milk. *Die Reinigung und Desinfektion in der Milchwirtschaft* (*Cleaning and Disinfection in the Dairy Industry*), by Mohr, Junger & Ritterhoff (2) is a most comprehensive book covering all aspects of the cleaning and sterilizing of dairy equipment; an appendix describes the stringent tests required by the German Agricultural Society for a certificate of approval of a detergent or disinfectant. A Ministry of Agriculture, Fisheries and Food Advisory Leaflet (No. 422) (3) has been published on *Sterilizing Farm Dairy Utensils with Approved Hypochlorite*. Davis (4) has reviewed the subject of cleaning and sterilizing in the dairy industry, and Sykes (5) on disinfection deals particularly with methods and modes of action. A summary of the proceedings of a Symposium (6) on methods for determining bacterial contamination on surfaces has been published in America, and Damm (7) has reviewed the chemical constitution of certain surface-active compounds and their specific action on micro-organisms of different types.

A useful paper from Germany (8) gives data on the characteristics, chemical composition, pH and surface tension at 20° C. in working dilutions, disinfecting power, corrosive effects on various metals, and suitability in the dairy industry of fifteen commercial detergents and disinfectants.

The advantages over conventional methods of in-place cleaning of dairy installations have been discussed by Holland (9), one of the great savings being, of course, on labour (10); this is well illustrated by Seiberling's (11) description of a plant consisting of 250 ft. of stainless-steel pipe-lines and fittings, two centrifugal pumps, an H.T.S.T. plate heat exchanger, a holding tube and a homogenizer, prepared for cleaning in 30 min. by one man, the whole cleaning operation taking only 75 min. Little difference in the bacteriological efficiency of in-place and standard take-down cleaning methods has been reported (12), though Flora, Arnold & Finnegan (10) found the bacterial counts of milk to increase less on passing down pipe-lines cleaned in-place than in those dismantled for cleaning. Studies in which a variety of detergents were circulated through 388 ft. of Pyrex glass pipe-line (12) showed much more variation between the efficiency of different detergents than between differing circulation temperatures for the same detergent. Kaufmann, Andrews & Tracy (13) have studied in particular the question of cleaning joints in pipe-lines; this problem may be eased by an American improvement of the in-place cleaning technique in which detergent solutions are drawn automatically through the pipe-lines in both directions alternately, turbulence being produced by a pulsating device (14). For bacteriological evaluation of the different cleaning methods and agents a brush and rinse test of milk contact surfaces gives consistently higher counts than a simple swab contact method (15). Resuggan & Davis (16) have shown that detergent solutions, containing up to 2·0% NaOH with or without added hypochlorite, used for 3–12 days in commercial bottle-washers frequently contain considerable numbers of bacteria; such solutions could be kept sterile for at least 6 days by the addition of a non-foaming quaternary ammonium compound (dioctyldimethyl-ammonium bromide).

The field of disinfectants has been reviewed in some detail by Elliker (17), who deals with the characteristics of hypochlorites, quaternary ammonium compounds (Q.A.C.), iodophors and others, and the factors affecting their bactericidal activity, such as the addition of wetting-agents to hypochlorites and of phosphates to Q.A.C.'s. The modes of action, relative efficiencies against vegetative cells, spores and bacteriophage, and the application of these principles in dairy and food processes are discussed. Retention by milk containers of small quantities of disinfectants used for rinsing has been studied by Seelemann & Wegener (18), who found a retention rate of 0·0018–0·0005%; this small quantity had no apparent effect on the bacterial content, keeping quality, pH or reductase activity of milk placed in the containers, and imparted no smell or taste. Lazarus (19) has given an account of iodine bactericides in the dairy industry, and considers that they compare favourably with chlorine and Q.A.C.'s. In this connexion Mueller (20) has shown that two commercial iodine cleaner-sterilizers at concentrations of 25 p.p.m. available iodine compared favourably with hypochlorite (100 p.p.m. available chlorine), though neither was effective against spores of *Bacillus cereus*. It has also been claimed (21) that milk cans cleaned with detergent solution can be effectively sterilized by ultra-violet irradiation, which may be cheaper than other sterilizing processes. An excellent paper by Clegg (22) gives details of three tests used for the evaluation of chemical sterilizers for dairy farms; the can test was used as a screening test, the surface film technique as a laboratory test, and farm trials gave a comparison between the bacteriological quality of milk produced using the standard control material (sodium hypochlorite with an alkaline detergent) and the test material; the finding that micro-organisms in liquid suspension are destroyed more rapidly than in a surface film (23,24) was confirmed. Other procedures for evaluating the efficiency of bactericidal agents are described by Taft (25), Chambers (26) and Stedman, Kravitz & Bell (27). The degree of water hardness influences to a marked degree the bactericidal efficiency of Q.A.C.'s, hard water being deleterious particularly because of bicarbonates, sulphates and chlorides of calcium and magnesium; ferrous, sodium and potassium carbonates are of much less significance. Convincing work to this effect has been done by Chambers, Kabler, Bryant, Chambers & Ettinger (28), but others have found, under their conditions, that hard water has no apparent adverse effect on Q.A.C. activity (29). Highly detailed

studies have been made by Ovejero (30) of the disinfectant properties of two Q.A.C.'s under various conditions of time, temperature and concentration, against a variety of micro-organisms, in particular *B. circulans* and *B. cereus* spores. Recent work reviewed by Resuggan (31) indicates that, in the concentrations used as sterilizing agents in the food industry, Q.A.C.'s are unlikely to have any toxic effects. Hoy & Neave (32) have continued their work on the disinfection of milk cans with hypochlorite, confirming earlier observations that failure is often due to the internal physical condition of the cans, rusty and rough surfaces affording some measure of protection for bacteria which are then not destroyed by solutions of much higher concentration than those reported to be effective under laboratory conditions; in fact, the physical condition of cans frequently has a greater effect than the type of disinfectant, the pH of the solution or the method of application. Seelemann & Rackow (33) have made comparative tests of two chlorine disinfectants used with a separate detergent, and two combined detergent-disinfectants, finding the latter to be less reliable, and to have a lower safety margin, in hot weather; this may have been due to their chlorine content. An interesting point by Ljunggren (34), of considerable practical importance, is that the sensitivity of Wode's test for free chlorine in milk is appreciably affected by the form in which the chlorine is added; a lower concentration of free chlorine was needed to give a positive reaction with hypochlorite than when chloramine was added to the milk. In studies of the effect of hypochlorite on the bacterial flora and bacterial numbers in milk McKenzie & Booker (35,36) found that micrococci were more susceptible than streptococci, and that less than 50 p.p.m. available chlorine had little effect on acid production by lactic streptococci. It is interesting that no bactericidal effect was apparent within 15 min. of adding hypochlorite to milk, even at concentrations of 500 p.p.m. available chlorine; however, after storage at room temperature for 24 hr. a marked reduction in numbers occurred with only 10 p.p.m.

As in previous years the cleaning and disinfection of milking machines has received considerable attention, the treatment of rubber components in particular; the value of the immersion cleaning technique (37), despite its recent inception, is fast becoming clear. Thiel *et al.* (38) have described a year's trials carried out on eight commercial farms, four using a 5% and four a 1% caustic soda solution for immersion cleaning, the results showing that milk of good bacteriological quality could be produced consistently with either concentration. However, high counts were sometimes obtained with cluster rinses, particularly when the teat-cup liners were old, suggesting some bacterial protection, and the need for improved bactericidal action, at the rubber surface. This was also encountered by Tjøtta & Solberg (39) who immersed teat-cups and liners in detergent followed by disinfectant solutions, and subsequently found deposits and cracks on the liners; however, Thiel (40) has shown that the addition of ethylene diamine tetra-acetic acid to caustic soda solution prevents lime deposits, whether from water hardness or milk calcium, and thus gives improved bactericidal action at the liner surface. Carreira *et al.* (41) found no marked differences in the bacteriological quality of milk produced under immersion cleaning and hot detergent with hypochlorite or steam sterilization régimes; they found the cow's udder to be the main source of micro-organisms in the milk, satisfactorily cleaned utensils contributing very few, and that the flora of immersion-cleaned equipment consisted predominantly of micrococci. Clegg (42) has reviewed important aspects of the chemical sterilization of milking machines, including official regulations, while Kosikowski & Holland's (43) pamphlet describes the cleaning and sterilizing of milking machines, in-place cleaning by both the circulation and reverse-flush systems in particular; they include many good photographs illustrating the various procedures. The advantages of recirculation cleaning have been emphasized by various workers (44,45,46), the labour-saving aspect being enhanced still further by the use of permanent glass pipe-lines (47). Bacterial contamination of teat-cup liners and other rubber parts of milking assemblies following deterioration of the inside surfaces has also been the subject of a number of papers, the danger from old, worn liners being greater the higher the bacterial counts of the milk passing through them (48). Claydon (49) found lower bacterial counts with new plastic than with new rubber, and also showed, like Noring (50), that differences in bacterial count between new and used rubber liners were much reduced when all the liners were wet-stored in lye solution. Although wet-

storage remains popular there is still no united opinion as to the best agent for this purpose (51,52), in terms of antibacterial efficiency and preserving the life of the liners; in the latter connexion, profuse bacterial growth on vulcanized rubber is not always associated with attack on the rubber hydrocarbon molecule, though Rook (53) has shown that several strains of *Streptomyces* do have this property, forming definite holes in thin rubber strips. In comparative tests against sodium hypochlorite and a Q.A.C. Stewart, Spencer, Lasmanis & Holliday (54) evaluated a new disinfectant, Chlorhexidine (bis-*p*-chlorphenyl-diguauidohexane diacetate), for teat-cup decontamination, using liners artificially infected with milk suspensions of various mastitis pathogens; chlorhexidine compared favourably in both bacteriostatic and bactericidal effect and had a high activity in the presence of organic matter.

VII. PASTEURIZATION

The objects and fundamental means of effecting processes such as pasteurization and sterilization may frequently be lost sight of in a maze of regulations and technical data; in *The Principles of Milk Legislation and Control*, mentioned on p. 400, Lethem (1) deals with these matters, and the phosphatase test, refreshingly clearly and simply, although briefly. J. G. Davis (2) has written a comprehensive and well-illustrated book on the *Laboratory Control of Dairy Plant* whose scope is reflected in a bibliography of some 900 references. The principles of the thermal destruction of micro-organisms have been set out by Collins & Dunkley (3) who call for caution with high-temperature no-hold processes in view of the dangers in extrapolating data obtained at low treatment temperatures; instrumentation and control of such high-temperature pasteurization processes have been discussed by Robinson (4), and equipment of German origin by Thomsen (5). The survival of micro-organisms in milk and dairy products has been reviewed by Engley (6) and Smith (7), the latter dealing specifically with pathogens. Lampe (8) has considered the various milk pasteurization techniques, and has critically reviewed the published literature, and the Report (9) of a Canadian Committee on Applied Laboratory Methods contains several sections of considerable interest in various aspects of pasteurization. Read, Boyd, Litsky & Norcross (10) have now published an illustrated description of the design, construction and operation of their laboratory apparatus for the 'come-up-time' method of milk pasteurization, and also details of bacteriological studies with this process (11), including the determination of time-temperature combinations giving 99·9% destruction of various bacteria and the effect of different heat-treatments on protein, phosphatase, flavour, pH and cream line (12). The elimination of bacteria from milk by centrifugation has been discussed elsewhere (p. 400), but it should be mentioned here that in order to test the method on an industrial scale under commercial conditions Simonart, Poffé & Wuytack (13) installed in a commercial dairy an ultra-centrifuge of 3000 l./hr. capacity capable of developing 9100 *g*. In some sixty tests involving more than 3000 gal. of bulk milk an average of 92·9% (86·3–97·6%) of the bacteria present were eliminated; the nature and significance of the residual organisms may, of course, be critical factors.

The close agreement between the results of the Kay–Graham and Aschaffenburg–Mullen phosphatase tests for the control of milk pasteurization is now well substantiated (14,15,16); the latter is to be preferred with flavoured milks (16), which frequently have enhanced phosphatase activity compared with plain milk (17), as it is not affected by the benzoic acid frequently used as a preservative for the colouring and flavouring ingredients. Modifications of these and other phosphatase test methods continue to be sought in the interests of improved sensitivity (18,19,20). The phosphatase enzyme is also enriched in cream and Fay (21) and Trembath & Newman (22) have stated that a more severe heat treatment is required for cream than for milk to produce phosphatase inactivation, and have reported positive phosphatase results for cream submitted to time-temperature treatments that were satisfactory for milk. It has likewise been tacitly assumed that the destruction of *Mycobacterium tuberculosis* requires more heat in cream than in milk. To determine these points by direct experiment Aschaffenburg, Briggs, Crossley & Rothwell (23) artificially infected with *M. tuberculosis* 'single' and 'double' (18 and 48% fat) cream,

which they then pasteurized by both Holder and H.T.S.T. methods at various time-temperature combinations. Kay–Graham and Aschaffenburg–Mullen phosphatase determinations, and guinea-pig tests, made on the treated creams showed that *M. tuberculosis* and phosphatase are as readily inactivated by heat in cream as in milk; in fact, *M. tuberculosis* did not survive H.T.S.T. or Holder pasteurization for the minimum times (15 sec. and 30 min., respectively) at temperatures 6° F. below the minima prescribed for milk. Aschaffenburg *et al.* were fortunate in encountering, also, a naturally occurring *M. tuberculosis* infection in one of their batches of cream, which greatly enhanced the value of the results obtained. Theoretically, there seems thus to be no reason why the minimum requirements prescribed for the pasteurization of milk should not be applied to cream. However, while compliance could be ensured at the plant through the application of the phosphatase test to the fresh product, the enzyme is known to reactivate frequently during storage of H.T.S.T. (though not Holder) pasteurized cream, magnesium and/or zinc ions playing some part in reactivation (24). The phosphatase test cannot, therefore, be applied to pasteurized cream samples of unknown age and history, and control must still depend as yet on technical efficiency of the processing plant (23). The development of a control test suitable for aged samples of pasteurized cream merits urgent attention since practically all market cream, at least in Britain, is subjected to some form of heat treatment, usually H.T.S.T., prior to retail sale, and figures (for milk) show the H.T.S.T. method to be the most satisfactory in terms of the destruction of *M. tuberculosis* (25); as Lethem (1) points out, 'no regulation' (for cream pasteurization in this instance) 'should be made which cannot be enforced'.

As in previous years a very considerable volume of literature has appeared on the keeping quality of pasteurized milk (26,27), and the various organisms responsible for its deterioration (28, 29,30). In fact, it sometimes seems that part of the attention paid to this aspect of pasteurization might, perhaps, be profitably applied to problems of clean milk production and efficient transportation, before the question of pasteurization is involved at all—a point very well made by Bleier (31). However, post-pasteurization contamination may be, of course, as important, or even more so, than the original milk flora, in determining keeping quality (32); tests for post-treatment contamination are not yet all that could be desired (33), although Winther (34,35) stresses the value of plate counts and coliform counts in conjunction with reductase tests for distinguishing between initial contamination of the raw milk and after-contamination from the plant, after-infection being indicated by high counts and *Bacterium coli* levels accompanied by short reduction times. Other work on keeping quality tests has been published by Olsen (36), Malling Olsen (37,38), Day & Doan (39,40) and, specifically on the detection of coliform contamination, by Frayer (41) and Wilssens & de Vleeschauwer (42). Williams (43,44) has done considerable detailed work on the growth characteristics and associative growth of various thermoduric bacteria in liquid media and in pasteurized milk, and their effects on tests for the keeping quality of both raw and pasteurized milk. Olson, Parker & Muelier (45) have reviewed work on the detection, nature, significance and control of psychrophilic bacteria in raw and pasteurized milk and other dairy products.

In-bottle pasteurization of milk has been reviewed by Crossley (46), who gives an account of its history, advantages and disadvantages, and discusses the legal aspects of the process. Cartons are rapidly increasing in popularity for the packaging of pasteurized milk, one of the best forms from both hygienic and economic points of view being the 'Tetra-Pak' (47) which, from the bacteriological standpoint, compares very well with glass bottles (48,49,50); Masoni, Zuliani, di Bella & Sbrozzi (51) have shown by artificial contamination of the outer surfaces of milk containers with *Serratia marcescens* and *Pseudomonas aeruginosa* that Tetra-Pak cartons are considerably less susceptible to extraneous contamination than conventional foil-capped glass bottles.

Other processes

Reviews have appeared on the different methods and equipment available, particularly high-frequency current and irradiation, for the sterilization of tinned foods, including dairy products (52), and on the preservation of such products by low-temperature storage and antioxidants (53); the

latter contains an interesting consideration of the importance of natural antioxidants such as tocopherols, lecithin and ascorbic acid and its esters.

A complete number of *Annales de la nutrition et de l'alimentation* (54) has been devoted to the proceedings of a series of meetings on various aspects, including technique and bacteriology, of sterilized milk; a new F.A.O. publication (55) covers similar ground, as well as the question of nutritive value. Although there have been misgivings on this score (56) the results of Mancini's (57) extensive experiments on the growth and reproduction of over 500 rats suggest that the nutritive value of sterilized milk is practically identical with that of pasteurized milk, though Hermier & Mocquot (58) state that much of the lactalbumin, but none of the lactoglobulin, in milk is denatured. However, this latter point is hard to accept since it is known that, under the influence of heat, globulin is the first to suffer (before albumin) and, on the immunological side, the so-called immune globulins in milk (and especially in colostrum) are certainly affected first. Even though Hermier & Mocquot's treatment times at temperatures over 100° C. were only fractions of a second, the milk had been preheated to 80° C. which would certainly be expected to affect globulin. Much of the recent work on the bacteriology of sterilized milk and ultra-high-temperature sterilization of milk (see later) has been on aerobic spore-forming organisms, many of which are, of course, responsible for spoilage serious in both incidence and degree, some also being highly heat-resistant. Burton, Akam, Thiel, Grinsted & Clegg (59) observed a build-up of thermophilic spores in commercial batch sterilizers, which may well be explained by the finding of Grinsted & Clegg (60) that thermophilic spore-formers (e.g. *Bacillus calidolactis*) sporulate very rapidly compared with mesophilic varieties such as *B. subtilis* and *B. licheniformis*. Franklin, Williams & Clegg (61), Candy & Nichols (62) and Ridgway (63) have studied in considerable detail the incidence, concentration and type of spore-formers in sterilized milk, confirming, and agreeing closely on, the preponderance (70–90%) of *B. subtilis* and *B. licheniformis* over other varieties. Ridgway and Candy & Nichols observed seasonal variation in spore counts, though Franklin *et al.* found no quantitative or qualitative differences. *Bacillus* species have also been incriminated in the spoilage of condensed milk (64, 65). Well worth the attention, on more fundamental grounds, of those interested in spore-bearing organisms is Schmidt (66) on 'the resistance of bacterial spores with reference to spore germination and its inhibition'; of potential practical application are Taylor's (67) observations that milk 'sterilized' in the absence of oxygen shows greater chemical changes, and is a much less favourable medium for the germination and growth of (*Bacillus*) spores, than milk so treated under aerobic conditions. Clegg (68) has surveyed the defects commonly occurring in commercially pasteurized and sterilized milks, and has made out the case for ultra-high-temperature sterilization (U.H.T.), discussing the possibilities of producing a sterile milk by means of U.H.T. treatment with aseptic filling (69). In this connexion Williams, Burton, Franklin & Clegg (70) have compared in a commercial dairy the effects of normal pre-heating (175° F.) with those of U.H.T. 'pre-heating' (285° F.) prior to bottling and sterilization at 225° F. for 35 min., U.H.T. pre-heating resulting in a striking improvement in the bacteriological quality of the sterilized milk, and having no effect on colour (as determined by reflectance measurements (71)) or on flavour. Milk bottles are a serious source of contamination by spore-formers (72), and sterility of containers, and aseptic filling of them, are important considerations in the U.H.T. milk sterilization process; using a new bottle-testing technique Franklin & Clegg (73) found 35% of bottles test-cleaned in a commercial bottle-washer in a sterilized milk factory to be contaminated with spores, and Galesloot (74) has described a method of estimating treatment efficiency using test milk inoculated with *B. subtilis* spores.

Other means and methods of preserving milk that have been reviewed or studied specifically of late include high-frequency electricity (75), ultrasonic waves (76) (whose effect may be purely thermal (77)), homogenization and freezing (78, 79), and ultra-violet irradiation (80) which is of value in *Bacillus* spp. spoilage of condensed milk (64, 65). A list (81) has been published of all the naturally-occurring and synthetic materials used as preservatives in American food processing and dairy products, and Lück (82) has reviewed the use of hydrogen peroxide in dairying.

Ice-cream

The bacteriological aspects of the manufacture of ice-cream have been reviewed by Storgårds (83) who covers sources of infection, pasteurization and hygienic measures, and methods of bacteriological control. The work of Speck, Grosche, Lucas & Hankin (84) on H.T.S.T. pasteurization of ice-cream mix using as test organisms heat-resistant *Microbacterium* and *Micrococcus* species was mentioned in the last Review (85) of this series; Tobias, Kaufmann & Tracy (86, 87) have now published similar work, with *Micrococcus* strain MS102, showing that 187·2° F. for 0·8 sec. (estimated effective holding time) gives destruction equivalent to pasteurization at 155° F. for 30 min. They calculate for *M. tuberculosis* from the data obtained with *Micrococcus* that, in order to maintain the margin of safety at present afforded in commercial pasteurization, the processing temperature would need to be raised to 194° F. (/0·8 sec.); it is regrettable, however, that direct experimentation along these lines has yet to be made with *M. tuberculosis* itself. Siegmund (88) has shown that foam from the surface of vat-pasteurized (165° F./30 min.) ice-cream mix frequently harbours viable coliform organisms, likewise the foam of mix heated in a tubular heater and kept at pasteurizing temperature in holding vats, and even that of H.T.S.T. treated mix in which air had been purposely entrapped before treatment. The tests commonly used for the bacteriological control of ice-cream have been discussed by Cominazzini (89); in connexion with coliform determinations, Barber & Fram (90) have found that, although ice-cream made from fruit juices generally has a lower bacterial count than that made from milk (91), fruit ice-cream and the fruits used in its manufacture are frequent sources of coliform false-positives; the organisms involved were not fully identified. Mossel, Mandersloot & Gentis (92) have developed a test for chemical preservatives in milk and ice-cream based on the inhibitory effects of the additives on gas production by *Saccharomyces cerevisiae*; the test is more sensitive than their original method using *Bacterium aerogenes* (93), but fails to detect low levels of chlorine-producing agents.

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